White matter correlates of Conduct disorder and developmental psychopathy

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List of abbreviations

5HT	5 Hydroxytryptamine
11ß-HSD-2	11 Beta-hydroxysteroid dehydrogenase-Type 2
ACTH	Adrenocorticotropic hormone
ADHD	Attention Deficit Hyperactivity Disorder
ANOVA	Analysis of Variance
AO	Adolescence-onset
APA	American Psychiatric Association
APSD	Antisocial Process Screening Device
ASC	Autism Spectrum Condition
ASD	Autism Spectrum Disorder
ASPD	Antisocial Personality Disorder
AUD	Alcohol use disorder
AVP	Arginine Vasopressin
BOLD	Blood Oxygen Level-Dependent
CD	Conduct disorder
CNS	Centre for Neuroimaging Sciences
CORT	Cortisol
CP	Conduct problems
CRF	Corticotropin Releasing Factor
CS	Conditioned Stimulus
CU	Callous-unemotional
D_{parr}	Parallel Diffusivity
D_{perp}	Perpendicular Diffusivity
DSM-IV-TR	Diagnostic and Statistical Manual of Mental Disorders – Third Revision
DT-MRI	Diffusion Tensor Magnetic Resonance Imaging
DWI	Diffusion Weighted Image
EHI	Edinburgh Handedness Inventory
EO	Early-onset

EMG	Electromyography
EPDS	Edinburgh Postnatal Depression Scale
EPI	Echo Planar Image
ESPAD	European school Survey Project on Alcohol and other Drugs
FA	Fractional Anisotropy
fMRI	Functional Magnetic Resonance Imaging
FSIQ	Full Scale Intelligence Quotient
FSL	Fmrib Software Library
GHB	Gabba-hydroxybutyric acid
GR	Glucocorticoid Receptor
HPA	Hypothalamic-Pituitary-Adrenal
ICD-10	International Classification of Diseases
IFOF	Inferior frontal occipital fasciculus
ILF	Inferior longitudinal fasciculus
IQ	Intelligence Quotient
K-SADS-PL	Kiddie Schedule of Affective Disorders and Schizophrenia – Present and Lifetime
LabTAB	Laboratory Temperament Assessment Battery
Log	Log transformed
McDESPOT	multicomponent driven equilibrium single pulse observation of *T*1/*T*2 - Myelin mapping scanning sequence
MD	Mean Diffusivity
MNI	Montreal Neurological Institute
mPFC	Medial Prefrontal Cortex
MR	Mineralocorticoid Receptor
MRI	Magnetic Resonance Imaging
nM/L	Nanomoles per litre
ODD	Oppositional defiant disorder
OFC	Orbitofrontal Cortex
PCL-YV	Psychopathy Checklist – Youth Version
PCL-R	Psychopathy Checklist - Revised
pCRH	Placental Corticosteroid Releasing Hormone
PFC	Prefrontal Cortex

PIS	Patient Information Sheet
PRU	Pupil Referral Unit
RF	Radio Frequency
ROI	Region of Interest
RR	R-top to R-top interval
SCR	Skin Conductance Response
SD	Standard Deviation
SDQ	Strengths and Difficulties Questionnaire
SES	Socio-economic status
SLE	Stressful Life Events
SLF	Superior Longitudinal Fasciculus
sMRI	Structural Magnetic Resonance Imaging
SPSS	Statistical Package for the Social Sciences
SVM	Support Vector Machine
TBSS	Tract-Based Spatial Statistics
TE	Echo time
TR	Repetition time
UF	Uncinate Fasciculus
US	Unconditioned Stimulus
VBM	Voxel Based Morphometry
vmPFC	Ventromedial Prefrontal Cortex
WASI	Wechsler Abbreviated Scale of Intelligence
WM	White matter
YOT	Youth Offending Team
Z_{obs}	Z observation

Abstract

Conduct disorder (CD) is a serious disruptive behaviour disorder that is diagnosed in children who display repetitive and persistent antisocial behaviour, such as violence, robbery and vandalism. Children with CD present substantial costs to society, and are the group of children most commonly referred to mental health services. Further, CD is a strong predictor of adult Antisocial Personality Disorder and psychopathy. Research to date on the biological associates of CD has mostly compared the anatomy and function of specific brain regions in people with CD to controls. However, there is increasing recognition that brain regions do not act in isolation. Rather, they form part of integrated neural systems. Nevertheless, to date, there have been no studies on anatomical 'connectivity' in CD.

Also there are few studies of how prenatal environment modulates the development of human limbic 'social brain' regions that are implicated in CD, and other abnormalities in social development. For example, prior studies reported behaviour problems in babies and children of mothers with elevated levels of stress or anxiety during pregnancy. Preliminary evidence suggests that these maternal emotional factors modulate intrauterine environment (e.g. through the stress hormone cortisol); and so may alter the development of limbic brain structures (such as the amygdala and orbitofrontal cortex) that are crucial to emotion processing and social cognition. However, to date, only one human study has examined the association between prenatal maternal mood and altered development of neural systems in children.

Diffusion Tensor Magnetic Resonance Imaging (DT-MRI) enables the microstructural integrity of white matter tracts to be quantified, providing a proxy measure of anatomical 'connectivity'.

Therefore, this thesis presents three DT-MRI investigations of the microstructural integrity of white matter tracts in (a) a population of adolescent males with CD and healthy control participants and (b) a group of children with known antenatal maternal stress levels and measures of *in utero* cortisol concentration. Study 1 used DT-MRI tractography to investigate fronto-limbic 'connectivity' in CD compared to controls within a specific fibre tract implicated in the pathophysiology of adult antisocial behaviour (the uncinate fasciculus) but never before investigated in younger individuals. I found abnormal fronto-limbic tract integrity in CD compared to controls, as indexed by significantly increased fractional anisotropy and reduced perpendicular diffusivity. Further, in healthy boys there was a significant correlation between measures of tract integrity and increased age, whereas boys with CD did not show this pattern of normal development. Study 2 determined if these findings were regionally specific by exploring whole-brain white matter microstructure in CD individuals and controls. This approach revealed between-group differences in several white matter tracts connecting the cerebellum, brainstem and cortical regions – pathways that have been implicated in aggression and affective processing by prior studies of animal models and non-CD populations. Study 3 investigated whether differences in white matter integrity of the uncinate fasciculus (found to be abnormal in CD in Study 1) is associated with variations in maternal

antenatal stress. I found significant relationships between antenatal maternal stressful life events and *in utero* cortisol concentration and fronto-limbic white matter microstructural integrity in children at age 6-9.

Together these studies demonstrate that adolescents with CD have abnormal white matter tract development, and suggest that prenatal events may modulate the development of some of these pathways. Future longitudinal studies are needed to confirm these findings and to explore factors that may ameliorate this developmental process. The final chapter of the thesis provides an overall summary of my findings, and considers future directions for research leading on from these studies.

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Finally, thank you to my parents for their continual support in all that I do.

I dedicate this thesis to the memory of Christopher John Evans: never far from my mind; forever an inspiration.

Contributions

I carried out all data collection and analysis presented in the thesis. Further, I carried out all the recruitment for Study 1. Participants in Study 2 were from an existing cohort set up by Imperial College London in 2001. I recruited these participants for my study with the assistance of Fiona Rose-Clarke, a part-time Research Assistant.

1: Antisocial behaviour in children

Current classification systems list two childhood antisocial behaviour disorders: Oppositional Defiant disorder (ODD) and Conduct disorder (CD).

1.1 Oppositional Defiant disorder

ODD is listed in the Diagnostic and Statistical Manual of Mental Disorders (DSM-IV-TR; American Psychiatric Association 2000) as a behavioural disorder that may be evident from as early as 5 years old, in the form of:

"…negativistic, hostile, and defiant behavior [sic] lasting at least 6 months, during which four (or more) of the following are present:

Table 1.1: DSM-IV-TR Oppositional Defiant disorder criteria

often loses temper
often argues with adults
often actively defies or refuses to comply with adults' requests or rules
often deliberately annoys people
often blames others for his or her mistakes or misbehavior
is often touchy or easily annoyed by others
is often angry and resentful
is often spiteful or vindictive"
(APA 2000)

ODD is diagnosed when a child's difficulties cause functional impairments, if the behaviours are in excess of levels found in his/her peers, and where diagnostic criteria for Conduct disorder (see below) are not additionally met. The World Health Organisation International Classification of Diseases (ICD-10; WHO 2004) which also contains this diagnostic category, states that this is a diagnosis found usually in younger children. Similarly, the earlier DSM-IV (APA 1994) noted that mean onset occurs before 8 years old.

Table 1.2: ICD-10 Oppositional Defiant disorder criteria

"Conduct disorder, usually occurring in younger children, primarily characterized by markedly defiant, disobedient, disruptive behaviour that does not include delinquent acts or the more extreme forms of aggressive or dissocial behaviour."
(WHO 2004)

The prevalence of ODD has been estimated as between 2% and 11% (Simonoff, Pickles et al. 1997; Nock, Kazdin et al. 2007); and studies have generally reported minor (Maughan, Rowe et al. 2004) or no (Lahey, Schwab-Stone et al. 2000) difference in rates of oppositional behaviour between boys and girls. A lack of consensus over prevalence rates arises from, first, difficulties in the identification of ODD in pre-school children, for who a certain amount of oppositional behaviour is deemed normal (Rey and Walter 1999). Second, high rates of comorbidity exist between ODD and CD; the co-occurrence is reported as around 12% in 5-7 year olds, to around 60% in

adolescents (Maughan, Rowe et al. 2004), and up to 97% in clinical populations (Frick 1992). As ODD is considered to be a precursor or prodrome of CD by virtue of its symptoms being a mild extension of normal childhood behaviour (Nock, Kazdin et al. 2007), a diagnosis of CD overrides that of ODD (APA 2000); this makes epidemiological studies requiring a clear delineation between these disorders difficult.

Importantly, ODD is considered to be a significant risk factor for the development of CD (DSM-IV 1994; Burke, Loeber et al. 2005; Rowe, Costello et al. 2010). It is unclear what factors influence the continuation of ODD into adolescence, or its conversion into CD. However, it is known that while for the majority of children ODD does not develop into CD, most children diagnosed with CD will have previously exhibited ODD (Rey and Walter 1999; Matthys and Lockman 2010). It has been shown that ODD does not independently associate with the development of adult Antisocial Personality Disorder (ASPD), but nevertheless exerts a risk via its link to the development of CD (Diamantopoulou, Verhulst et al. 2010; Rowe, Costello et al. 2010). Thus ODD is an important clinical entity that is pertinent to any discussion of the development of adolescent and adult antisocial behaviour disorders.

1.2 Conduct disorder

1.2.1 Definitions

Conduct disorder is defined by the DSM-IV-TR as:

“A repetitive and persistent pattern of behaviour in which the basic rights of others or major age-appropriate societal norms or rules are violated”.

Of the criteria given in Table 1.3, at least three should have been exhibited within the past 12 months, and at least one in the past 6 months, for a diagnosis to be made.

Table 1.3: DSM-IV-TR Conduct disorder criteria

Aggression to people and animals
often bullies, threatens, or intimidates others
often initiates physical fights
has used a weapon that can cause serious physical harm to others
has been physically cruel to people
has been physically cruel to animals
has stolen while confronting a victim
has forced someone into sexual activity
Destruction of property

has deliberately engaged in fire setting with the intention of causing serious damage

has deliberately destroyed others' property (other than by fire setting)

Deceitfulness or theft

has broken into someone else's house, building, or car

often lies to obtain goods or favours or to avoid obligations (i.e., "cons" others)

has stolen items of nontrivial value without confronting a victim (e.g., shoplifting, but without breaking and entering; forgery)

Serious violations of rules

often stays out at night despite parental prohibitions (before age 13)

has run away from home overnight at least twice while living in parental or parental surrogate home (or once without returning for a lengthy period)

is often truant from school (before age 13)

(APA 2000)

Conduct disorder is not diagnosed in over-18s, as a diagnosis of ASPD would then apply; this is similar to the way that a diagnosis of CD supersedes that of ODD.

The ICD-10 classifies CD similarly to DSM-IV-TR, as:

“a repetitive and persistent pattern of dissocial, aggressive, or defiant conduct. Such behaviour should amount to major violations of age-appropriate social expectations; it should therefore be more severe than ordinary childish mischief

or adolescent rebelliousness and should imply an enduring pattern of behaviour (six months or longer)”

Table 1.4: ICD-10 Conduct disorder criteria

fighting or bullying

cruelty to other people or animals

severe destructiveness to property

fire-setting

stealing

repeated lying

truancy from school

running away from home

unusually frequent and severe temper tantrums

disobedience.

NB: Any one of these behaviours, if marked, is sufficient for the diagnosis, but isolated dissocial acts are not

(WHO 2004)

The ICD-10 provides three diagnostic sub-classifications of CD; these are: (1) confined to the family context – where behaviours are only exhibited within the household to members of the immediate family; (2) unsocialised CD – where the disorder is accompanied by severe difficulties in social interactions, and where children are solitary; and (3) socialised CD – where individuals have

healthy peer relationships and frequently engage in disordered behaviour as part of their social group. The DSM-IV-TR, on the other hand, does not classify on this basis, but instead subtypes CD by a variable shown to be of importance in determining the prognosis of CD, namely age of onset.

1.2.2 Prevalence

CD has been estimated to occur in between 6% and 16% of the population (Sholevar and Sholevar 1995; Olsson 2009), and its prevalence is thought to be rising (Tcheremissine & Lieving, 2006). CD occurs more commonly in males (Moffitt and Caspi 2001), with rates of 2-9% reported among females (Sholevar and Sholevar 1995). Young people with Conduct disorder are the most commonly clinically referred sample to child mental health services, with an estimated third to two-thirds of all Child and Adolescent Mental Health Service referrals being for CD (Richardson and Joughin 2002).

1.2.3 Impact and outcomes of Conduct disorder

Antisocial behaviour in childhood is associated with a significant financial burden. For example, children with severe CD cost society 10 times more to support into adulthood than children without CD (Moffitt 2001; Scott, Knapp et al. 2001; Foster and Jones 2005; Romeo, Knapp et al. 2006; Passamonti, Fairchild et al. 2010). Also youth crime/antisocial behaviour costs the UK taxpayer over £4 billion per year (Independent Commission on Youth Crime and

Antisocial Behaviour 2010). Further, CD is considered a risk factor for various psychiatric conditions beginning in adolescence or adulthood. For instance, 40-75% of children with CD grow up to have ASPD (Zoccolillo, Pickles et al. 1992b; Gelhorn, Sakai et al. 2007a; NICE 2010); and there is a strong association between CD, substance abuse (Kessler, Nelson et al. 1996) and mood disorders (Vloet, Konrad et al. 2008).

1.2.4 Comorbidity and Conduct disorder

The most common comorbid condition with CD is Attention deficit hyperactivity disorder (ADHD), a developmental psychopathology commonly first diagnosed in childhood. Rates of ADHD are estimated at between 2-12%, with a male to female ratio of between 2:1 – 9:1 (DSM-IV-TR 2000). ADHD involves a recurrent pattern of inattentiveness, or additional hyperactivity and impulsivity. Examples of inattentiveness include difficulties sustaining attention, and being easily distracted or forgetful. Hyperactivity symptoms include persistent and inappropriate running, climbing or fidgeting. These symptoms can result in significant difficulties for children at home, school or with peers. Furthermore, and in common with CD, the risks associated with ADHD include antisocial behaviour, anxiety and mood disorders, and substance use (Wilens, Biederman et al. 1999).

ADHD is estimated to co-occur with CD at greatly variable rates (between 35% and 90%) (Abikoff and Klein 1992; Offord, Boyle et al. 1992). Comorbid

individuals face increased risk of negative outcomes that exceed those associated with either of the disorders alone (Vloet, Konrad et al. 2008). Further, comorbid ADHD is associated with an earlier CD symptom onset (Loeber 1995). Finally, Johansson et al (2005) found that adult psychopaths were four times more likely to have had comorbid ADHD and CD in adolescence than either one, or neither, disorder. These studies illustrate the interconnected nature of ADHD and antisocial behaviour in children and adults. Moreover, they highlight that ADHD is an important confounding factor that needs to be accounted for when researching antisocial behaviour disorders.

1.3 Heterogeneity within Conduct disorder

1.3.1 Age of onset

The age at which CD arises is an important factor in determining the severity and developmental trajectory of the disorder. For example, the most common period during which CD develops is adolescence. However adolescent onset is associated with less aggressive and serious antisocial behaviour than earlier onset CD (Lahey, Loeber et al. 1998). Also, the risk of CD continuing into adulthood has been reported to be lower with an adolescent onset - being more likely to decrease after the teenage years (Farrington 1986). A diagnosis of adolescent-onset, or adolescent-limited, CD is made where disruptive behaviours begin after the age of 10 years old (DSM-IV-TR, APA 2000). In contrast, early-, or childhood-onset, CD begins prior to the age of 10, with the

majority of cases previously having met criteria for ODD (Matthys and Lockman 2010).

The age at which CD arises was first shown to be an important factor in determining the severity and developmental trajectory of the disorder by two key longitudinal studies (Moffitt 1993; Lahey, Loeber et al. 1998). These and later investigations found the sub-categories of childhood- versus adolescent onset to be related to distinct associations with regards to, first, the range of outcomes and, second, the different associated risk factors.

First, CD beginning before age ten is often associated with significantly poorer outcomes than a later onset of the disorder. For example, an early onset of CD is linked to greater levels of delinquency, aggression and violence (Kazdin 1995; Jeglum Bartusch, Lynam et al. 1997). Also, compared to a later onset, these children show a greater likelihood of their disorder persisting into adulthood and converting to ASPD (Frick & Loney, 1999). Although this has been named 'life-course persistent antisocial behaviour' (Moffitt 2003), the symptoms of approximately half of those with childhood-onset CD do not persist into adulthood (Robins 1978; Moffitt, Caspi et al. 1996).

Second, in terms of risk factors and other features, early-onset CD is associated with a number of familial, psychological and biological deficits and difficulties, which adolescent onset CD generally is not. These include low IQ (Fergusson, Lynskey et al. 1996; Piquero 2001), neurocognitive difficulties – such as low arithmetic skills, and lower verbal/non-verbal intelligence - (Donnellan, Ge et al.

2000) - that may be similar to those of children with left hemisphere brain damage (Golden and Golden 2001), and deficits in emotion processing (e.g. Fairchild, Van Goozen et al. 2009; described in Chapter 2). These findings led to the suggestion that early-onset CD constitutes a subtype that has a greater neurodevelopmental basis than adolescent-onset CD, which in contrast is proposed to be under a greater influence from social and environmental factors (Moffitt 1993; Moffitt, Arseneault et al. 2008). However, recent neuroimaging studies suggest that this may not be the case, as both subtypes of CD are found to be associated with abnormalities of brain anatomy and function (Fairchild, Van Goozen et al. 2009; Fairchild, Passamonti et al. 2011; see Section 3).

Therefore, despite childhood-onset CD being linked to increased risk for social, psychological, and biological difficulties, many studies do not subtype CD by age of onset. Instead, researchers have identified that a small group of children with CD show a 'callous-unemotional' temperament style (e.g. lacking empathy and guilt) from very early in childhood (Lynam and Lynam 2002; Sadeh, Verona et al. 2009) – and that it is the presence of this – rather than simply the age of onset of CD, that is proposed to delineate a more neurobiologically rooted subtype of CD. Also, some have suggested that having callous-unemotional temperament traits contributes to the development of early-onset CD (Viding, Hanscombe et al. 2010). The following paragraphs describe callous-unemotional traits and discuss the evidence for a neurobiological basis for CD and this type of temperament.

1.3.2 Callous-unemotional traits

Callous-unemotional (CU) traits are a distinct constellation of interpersonal and emotional characteristics that accompany disruptive or antisocial behaviour in approximately 25% of children with childhood-onset CD (Frick 1998). Traits include low fearfulness, impulsivity, shallow affect, poor empathy, and an absence of guilt (Hare 1991; Christian, Frick et al. 1997). Several assessment tools refer to CU traits as 'psychopathic' traits (see Methods Chapter 4). Nevertheless, CU and psychopathy are not equivalent constructs, as CU traits constitute only one of the three dimensions comprising 'psychopathic' temperament, the other dimensions being impulsivity and narcissism (Frick, Bodin et al. 2000; Cooke, Michie et al. 2006). Hence, although CU traits closely resemble those displayed by adults with psychopathy, the term 'psychopathic/psychopath' is not applied to children - both to avoid stigma and because of limitations in the current evidence base (Johnstone and Cooke 2007).

CU traits remain relatively stable throughout adolescence and into adulthood (Frick, Kimonis et al. 2003; Loney, Taylor et al. 2007; Lynam, Caspi et al. 2007b; Lynam, Loeber et al. 2008). Even early in life, having a high level of CU traits distinguishes youngsters from other conduct disordered children with regard to the onset and severity of their antisocial behaviour, and the risks of associated harm. For example, children with both CD and CU traits (as compared to those with CD alone) present with an earlier onset of their behaviour problems (Dandreaux, Frick et al. 2009), more severe behaviours

(Dolan 2004; Frick, Stickle et al. 2005), and have poorer outcomes - including substance use disorders, criminality and violent offending (Lynam & Gudonis, 2005).

Children with CU traits also differ from those with CD alone in both the amount and type of aggressive behaviour that they display. Based on early animal research two distinct subtypes of aggression have been delineated: 'affective-defence' versus 'predatory'; and this division has also been applied to humans (McEllistrem 2004). Affective aggression is that which is elicited through frustration, as a reaction to provocation, or in fearful response to a perceived threat (e.g. being attacked). Such aggression is instant and reflexive, and is accompanied by physiological arousal (Moyer 1968). Conversely, predatory aggression is defined as being premeditated and goal-directed; it is aggression that is targeted towards the reward of acquisition, or in order to assert dominance. This type of aggression does not involve emotional reactivity (Glenn and Raine 2009). In humans these aggressive subtypes are more commonly defined as reactive versus instrumental, respectively. While children with CD show high levels of the former (but not the latter), those with psychopathic traits show high levels of *both* reactive and instrumental aggression and violence (Kruh, Frick et al. 1999).

Although both types of aggression are elevated in children showing features of psychopathy, some studies have found instrumental aggression to be particularly associated with the CU dimension of psychopathy (Frick, Cornell et al. 2003a; Marsee and Frick 2007), and this may be related to biological

features of this temperament type. For example, the acquisition of CU traits has been attributed to a temperament of low behavioural inhibition and under-arousal resulting from aberrant autonomic nervous system reactivity (Lahey et al, 1993). This is exemplified by studies finding that children with high levels of CU traits show reduced autonomic (Anastassiou-Hadjicharalambous and Warden 2008; De Weid, Van Boxtel et al. 2012) and neural reactivity (Marsh, Finger et al. 2008a) towards negative emotional stimuli compared to those without CU temperament. In addition, CU populations show reduced behavioural responses to punishment cues during rewarded gambling tasks (Fisher & Blair, 1998). Taken together, these deficits suggest that children with CU traits have aberrant development of aversive conditioning (a type of emotional learning described in Chapter 2) to negative stimuli.

If so, this would imply the involvement of the amygdala and other parts of the limbic system in CU temperament - as they mediate emotional learning (Blair, Mitchell et al. 2005). There is preliminary evidence to support this suggestion (discussed further in Chapter 2, below). Further, these abnormalities may partially be explained by adverse antenatal environmental factors – such as exposure to stress or anxiety during foetal life – as these maternal mood states are associated with abnormal brain development in both animal (Uno, Eisele et al. 1994; Coe, Kramer et al. 2003; Salm, Pavelko et al. 2004; Kraszpulski, Dickerson et al. 2006) and human (Buss, Davis et al. 2010) offspring; and negative temperament and conduct problems in human infants and children (O'Connor, Heron et al. 2002b; Gutteling, de Weerth et al. 2005b).

The following chapters, therefore, review prior work on the neural correlates of child and adolescent conduct problems (Chapter 2); and the evidence for the influence of prenatal environmental factors on cognitive and behavioural neurodevelopment (Chapter 3). Together these sections provide the background that has led to the design of the subsequent experimental chapters of this thesis.

2: Neural correlates of childhood antisocial behaviour

Neuroimaging research has historically identified two main brain regions as being associated with antisocial behaviour and/or psychopathic traits: the amygdala, and the prefrontal cortex (PFC). To date most studies of antisocial behaviour have examined these two brain regions in isolation. However, through the use of advanced neuroimaging techniques, modern neuroscience informs us that complex social behaviours are not governed simply by individual brain structures. For this reason it is also important to examine interconnections *between* regions; these constitute neural networks. Therefore, this chapter will provide (i) a brief overview of the functions subserved by brain regions showing deficits in children with CD and/or CU traits; (ii) evidence for abnormality in those brain regions in these populations; (iii) an overview of the brain networks implicated in antisocial behaviour; and (iv) evidence for abnormal brain 'connectivity' in antisocial behaviour. Thus, the sections of this chapter aim to provide the rationale for the three studies that comprise this thesis.

2.1 Brain regions involved in antisocial behaviour

The two main brain regions that are associated with antisocial behaviour are the amygdala and the PFC. The functions of each of these two regions will first be described, followed by evidence from neuroimaging studies for their involvement in childhood antisocial behaviour.

2.1.1 The amygdala

2.1.1.1 Amygdala anatomy and function

The amygdala is an almond-shaped grey matter structure located within the medial temporal lobe; and it plays an important role in affective processing and emotional learning. Several experimental paradigms have been used to assess different aspects of amygdala function; some of these are described below alongside the evidence for amygdala dysfunction in childhood antisocial behaviour.

Figure 2.1: The amygdala

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Diagram of the amygdala (Myers 2000)

2.1.1.1.1 Emotion processing

The amygdala is vital for the recognition of negative emotional facial expressions (e.g. fearful facial expressions) and (in healthy individuals) it activates robustly in response to distress cues (Etkin, Klemenhagen et al. 2004), such as facial and vocal expressions of fear or sadness in others. Patients with amygdala lesions have deficits in the recognition of fearful expressions (Adolphs, Tranel et al. 1994; Adolphs, Tranel et al. 1995; Adolphs, Tranel et al. 1999; Papps, Calder et al. 2003). Further, in healthy individuals the amygdala is more active during the processing of fear and disgust, relative to happy and neutral, expressions (Phillips 2003; Costafreda, Brammer et al. 2008). Finally, the amygdala is important for the experience of fear; stimulation of the amygdala produces increased fearfulness in humans and animals (for review see Davis 1997), while bilateral damage is associated with fearless behaviours in humans (Feinstein, Adolphs et al. 2010).

Deficits in fear processing, such as those seen after amygdala damage, are also observed in individuals with antisocial behaviour. A recent meta-analysis of 20 studies of emotional processing in antisocial adults and children concluded that they show significant deficits in the recognition of fearful, sad, and surprised expressions – most significantly fear - in comparison to control groups (Marsh and Blair 2008). Also, adolescents show differential deficits in the recognition of facial expressions depending on the age of onset of CD. Specifically, compared to healthy controls, those with childhood-onset CD have deficits in the recognition of anger, disgust and happiness, whereas adolescent-

onset boys show a greater deficit for fear (Fairchild, Van Goozen et al. 2009). Finally, a study of emotion recognition within a community sample of boys with antisocial behaviour and CU traits found that high levels of CU traits were associated with significant deficits in the recognition of fearful faces (Dadds, Perry et al. 2006). However, antisocial behaviour scores were positively correlated with the tendency to misinterpret neutral expressions as angry - suggesting an increase in the perception of threat among boys with high levels of antisociality (Dadds, Perry et al. 2006). Thus, the overlap between the deficits seen in amygdala damaged populations and in antisocial and CU children has been taken to suggest that amygdala dysfunction plays a key role in the aetiology of these disorders.

Emotion recognition deficits in antisocial behaviour are also important when considering the way(s) in which distress cues (i.e. negative emotions displayed others) function as aversive stimuli. Distress cues should elicit empathic responses (e.g. feeling the distress of others) (Blair 1995; Blair, Mitchell et al. 2005) and contribute towards emotional learning (Hooker, Germine et al. 1996). In this view, amygdala dysfunction in children with CD, and especially those with CU traits, renders them insensitive to distress cues - which in turn affects moral socialisation (Marsh, Finger et al. 2008a). For example, the inability to pair the fear or sadness on somebody's face with feelings of guilt or empathy may lead to a failure to suppress behaviours that engender distress in others (ibid). Therefore, it has been suggested that antisocial behaviours in CD arise from a relative inability to empathise, and may consequently lead to abnormal socialisation (Blair and Blair 2003).

2.1.1.1.2 Affective modulation of startle reflex

Another emotion processing assessment that indexes amygdala reactivity is a paradigm that measures affective modulation of the startle reflex. In both animals and humans a reflexive eyeblink is elicited on the presentation of a startle probe, such as a loud burst of white noise. The reflex involves the sudden contraction of muscles below the eyes (the orbicularis occuli); the amplitude of this can be measured with electromyography (EMG) using electrodes placed below the eyes. The presentation of different valances of emotional stimuli (e.g. pictures) can modulate the amplitude of the startle reflex. In healthy people, compared to neutral images negative stimuli produce an enhanced eyeblink reflex, whereas positive stimuli produce a reduction. This difference is thought to reflect the internal emotional state being experienced, if this is negative or fearful the individual is primed towards threat. Thus, the startle probe should produce a larger reflex than during a pleasant mental state, where there is little threat (Fillon, Dawson et al. 1998). The involvement of the amygdala in this reflex has been demonstrated in animals, with extinction of this reflex occurring following lesions to the central nucleus of the amygdala (Hitchcock, Sananes et al. 1989). In humans, temporal lobectomy involving the left hemisphere results in reduced startle modulation to shock (Funayama, Grillon et al. 2001); and functional magnetic resonance imaging (fMRI) studies reported activation of the amygdala during the startle reflex (Anders, Lotze et al. 2004).

Abnormal eyeblink startle, similar to that found with amygdala damage, is also shown by children with antisocial behaviour. For example, Fairchild et al reported reduced eyeblink to positively and negatively valenced stimuli in a group of boys with CD as compared to healthy adolescents (Fairchild, Van Goozen et al. 2008a). Further, the authors found no significant difference in amplitude between early-onset and adolescent-onset boys - suggesting that the neurobiological deficits found were not associated with the age of onset of CD. Also, others reported that children with ODD have significantly reduced eye-blink startle as compared to controls, and that this correlates with the severity of conduct problems (Van Goozen, Snoek et al. 2004). In summary, taken together, this converging evidence further supports the suggestion that amygdala dysfunction may play a role in childhood antisocial behaviour.

2.1.1.1.3 Emotional learning

A further index of amygdala damage is disruption to emotional learning (LeDoux 2000; Rolls 2000; Baxter and Murray 2002). This type of learning serves to pair an aversive stimulus (e.g. punishment) with the resulting negative emotional outcomes (e.g. fear, guilt), which are thought to guide future behaviours and lead to moral socialisation (Blair, Mitchell et al. 2005). In a typical paradigm, a previously neutral stimulus (conditioned stimulus; CS) is paired with an aversive cue (unconditioned stimulus; US) so that on future trials the CS alone will become aversive. Studies in rodents with amygdala lesions first highlighted the role of the amygdala in this type of conditioning (e.g. Phillips and LeDoux 1992); the presentation of the CS fails to elicit the physiological reactivity (e.g. freezing,

increased heart rate) that arises from the US alone. Studies in humans have confirmed that damage to the amygdala and temporal lobe leads to deficits in emotional learning and fear conditioning (Bechara, Tranel et al. 1995; LaBar, LeDoux et al. 1995). Moreover, studies of *in vivo* brain responsivity as measured using fMRI revealed that the amygdala is activated during aversive conditioning (Buchel, Morris et al. 1998), thus illustrating the role of this structure in this function. This is of importance because emotional learning is modulated by aversive conditioning and deficits have also been reported in antisocial populations. For example, children with both early- and adolescent-onset CD have reduced skin conductance responses (SCR; an index of autonomic reactivity) during fear conditioning, as compared to healthy controls (Fairchild, Van Goozen et al. 2008a). This suggests abnormalities in the coupling of emotional stimuli with neural and/or autonomic responses in antisocial children.

2.1.1.1.4 Links to the hypothalamic pituitary adrenal (HPA) axis

The HPA-axis is the major system underlying the neuroendocrine stress response and is integral to the regulation of many vital bodily functions, including growth, metabolism, immunity, sexual function, mood, and emotion (Charmandari, Tsigos et al. 2005). This is of relevance to this thesis because one function of the amygdala is to facilitate communication between the limbic system and the HPA axis.

Figure 2.2: The HPA axis

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University of Montana (2011) *CRF – corticotropin-releasing factor; ACTH – adrenocorticotropic hormone*

Signals arriving at the paraventricular nucleus of the hypothalamus trigger the release of corticotropin releasing factor (CRF) and arginine vasopressin (AVP), which in turn promotes the secretion of adrenocorticotropic hormone (ACTH) from the anterior lobe of the pituitary gland. In response to ACTH, the adrenal cortex secretes cortisol, which binds to glucocorticoid and mineralocorticoid receptors (GR; MR) in the hypothalamus and pituitary gland to stem further release of CRH/ACTH through the action of negative feedback. These

receptors are expressed in both somatic and neural cells, explaining the far-reaching effects of cortisol on diverse bodily systems.

The amygdala plays an important role in the activation of the neuroendocrine stress response. Following threat detection, the central nucleus of the amygdala transmits signals to the hypothalamus; this activates the HPA-axis and culminates in cortisol secretion (Brown 2000). Damage to the amygdala in rats results in diminished secretion of corticosterone (the major stress hormone in rodents (Brown 2000)) after exposure to an acute stressor (Soloman, Jones et al. 2010). The amygdala is also highly sensitive to the actions of glucocorticoids as it contains abundant GRs. The significance of glucocorticoids in fear conditioning was illustrated in a mouse study which demonstrated that disruption to GRs in the central nucleus of the amygdala prevents fear conditioned responses (Kolber, Roberts et al. 2008). It is not clear, however, whether in humans amygdala dysfunction is related to a similar effect on cortisol secretion. Nevertheless, if this was the case then it may help explain reports that antisocial individuals have abnormalities in the anatomy and function of the amygdala together with reduced cortisol levels (the latter are summarised in Chapter 3).

In summary, there is evidence that children with antisocial behaviour have differences in the development of distributed brain systems involved in emotion processing and learning. Further, differences in emotion processing may contribute to the abnormal responding to distress cues observed in antisocial children. These differences may also account for the impairments in empathy,

and the use of instrumental aggression, seen in children with high levels of CU traits. Thus, the amygdala's role in both emotional processing and emotional learning may contribute to the behavioural deficits seen in severely antisocial populations. To test this idea, neuroimaging studies have assessed the anatomy and function of the amygdala of children with CD and/or CU traits; these findings are discussed below.

2.1.1.2 Correlates of amygdala dysfunction in antisocial children

2.1.1.2.1 Anatomical abnormalities

To date, three studies have revealed reduced amygdala volume in children with CD as compared to healthy children (Sterzer, Stadler et al. 2007; Huebner, Vloet et al. 2008; Fairchild, Passamonti et al. 2011); and this was also associated with empathy deficits in the CD group (Sterzer, Stadler et al. 2007). **T**hese studies highlight that anatomical abnormality of the amygdala may be underlying the emotional processing and affective learning deficits (outlined above) that are observed in children with CD.

2.1.1.2.2 Functional abnormalities

A number of studies report differences in the function of the amygdala in CD in comparison with healthy controls. fMRI studies of antisocial children with high levels of CU traits report that they have significantly reduced blood oxygen level-dependent (BOLD) activation to fearful faces in the right (Jones, Laurens

et al. 2009) or bilateral (Marsh, Finger et al. 2008a) amygdala. Further studies found reduced BOLD responses in the left amygdala to negative emotional pictures (Sterzer, Stadler et al. 2005), and bilaterally to angry facial expressions (Passamonti, Fairchild et al. 2010) in CD boys. Finally, the reverse pattern has been reported, with *increased* BOLD activation to negative stimuli in the left amygdala in a group of boys with comorbid CD and ADHD (Herpertz, Huebner et al. 2008). It is possible that the inconsistency between this and other findings results from the high level of comorbid ADHD. Nevertheless, enhanced activations of the amygdala, plus striatum and temporal areas, have also been reported within CD boys as they viewed scenes showing others experiencing pain (see below; Decety, Michalska et al. 2009). Increased signal in these areas was not shown in the control sample, despite both groups displaying similar activation increases within the pain matrix (comprising the anterior insula, medial cingulate cortex, somatosensory cortex, and periaqueductal grey). Taken together these fMRI studies illustrate that amygdala dysfunction is present in children and adolescents with antisocial behaviour and/or psychopathic traits. However, the *type* of difference (i.e. hyper-vs-hypo activation) may depend on the emotional valence of the stimuli.

2.1.1.3 Summary

To summarise, there is increasing evidence that children with antisocial behaviour and/or psychopathic traits have significant differences in the anatomy and function of the amygdala. Also, these abnormalities may be related both to

clinical features, and to underlying information processing deficits – such as impaired emotion recognition, empathy and associative learning.

2.1.2 Prefrontal cortex

2.1.2.1 Prefrontal cortex functions

2.1.2.1.1 Social behaviour

The second brain region found by many prior studies to be associated with antisocial behaviour is the prefrontal cortex (PFC). The importance of the frontal lobes to social behaviour was first recognised in the 19th century following the case of Phineas Gage, in whom frontal lobe damage resulted in profound personality change marked by inappropriate social behaviour (Harlow 1993 (1869)). A 'frontal lobe' syndrome was subsequently described based on clinical observation of the behaviour of patients with brain lesions (Lishman 1998). Characteristic features included apathy, emotional lability, lack of social awareness, unconcern for social rules, impulsiveness, and frustrative aggression (*ibid*). Such observations suggest that related traits in antisocial/psychopathic individuals may result from frontal lobe abnormalities (Damasio 2000).

Key regions of the human frontal lobe include the prefrontal cortex (frontal regions anterior to the motor cortices) and its subdivisions (anterior portions of

dorsolateral and medial cortices, and the orbital cortex). The ventromedial PFC (vmPFC) is an additional term used to refer to the orbitofrontal and/or the ventral portion of the medial wall of the frontal lobe (Stuss and Levine 2002). Damage to the vmPFC in particular is associated with an increased risk of reactive violence and aggression (Grafman, Schwab et al. 1996; Blair and Cipolotti 2000), and deficits in moral judgment (e.g. where vignettes detailing failed attempts at harming others were judged as more morally permissible by vmPFC lesioned patients compared to controls (Young, Bechara et al. 2010)).

2.1.2.1.2 Decision making

People with antisocial behaviour/psychopathy exhibit the same aberrant response pattern as patients with orbitofrontal damage on the Iowa Gambling Task (Bechara, Damasio et al. 1994; Mitchell, Colledge et al. 2002; van Honk, Hermans et al. 2002). This task requires individuals to choose cards from four decks of playing cards in order to receive rewards (financial gain) and avoid punishment (financial loss). The four decks are unequally weighted for losses and gains, with two decks consistently producing both high rewards and high losses and the other two small rewards and small losses. Over time healthy controls choose the latter two packs to produce a net gain. In contrast, those with vmPFC lesions show no bias towards these safer packs (Bechara, Damasio et al. 1994). This same deficit has been reported in CU children (Blair et al., 2001), students with sub-clinical psychopathy (van Honk, Hermans et al. 2002), and adult psychopaths (Mitchell, Colledge et al. 2002). Hence it has been suggested that abnormalities in the PFC, and particularly the vmPFC, play

a key role in mediating the social, emotional and cognitive deficits typically seen in CD and in those high in CU/psychopathic traits.

2.1.2.1.3 Links between frontal regions and the HPA-axis

As well as the PFC's involvement in social behaviour and decision making, it is also important for regulating the HPA-axis (Kern, Oakes et al. 2008). For example, recent work reported that PFC volume was inversely correlated with evening cortisol levels in adolescents (Carrion, Weems et al. 2010). Further, it has been proposed that the PFC and amygdala work together in responding to, and inhibiting, physiological threat responses involving the HPA axis (Urry, Van Reekum et al. 2006). .

2.1.2.2 Correlates of prefrontal cortex dysfunction in antisocial children

2.1.2.2.1 Anatomical abnormalities

While there is evidence for the role of the PFC in emotion, decision making and HPA-axis regulation, to date only two neuroimaging studies have identified anatomical abnormalities of this region in antisocial children. One study found reduced cortical thickness in the PFC, along with cingulate and insular cortices, in children with disruptive behaviour disorders as compared to healthy controls (Fahim, He et al. 2011). A further study showed increased grey matter concentration in the medial orbitofrontal, and anterior cingulate, cortices in a

group of children with elevated CU traits, compared to low CU scorers (De Brito, Mechelli et al. 2009).

2.1.2.2.2. Functional abnormalities

As well as these structural findings, fMRI studies have reported abnormal neural reactivity in the frontal lobe of antisocial children. Compared to healthy boys, adolescents with CD showed reduced BOLD activation in the vmPFC, orbitofrontal cortex, and insula, as well as the amygdala, to angry facial expressions (Passamonti, Fairchild et al. 2010). A further study found aberrant frontal lobe activation of the vmPFC in children with psychopathic traits during a reversal learning task (Finger, Marsh et al. 2008). This task requires inhibition of previously rewarded responses to avoid punishment from rule reversal errors. For example, a pattern of responding is established (e.g. pressing a button to stimulus A earns a reward, while pressing to stimulus B is punished). After a certain number of trials the contingency is reversed (i.e. A is now punished and B is rewarded). The vmPFC is crucial to successfully completing this task; and adult psychopaths have significant deficits in this (Fellows and Farah 2003; Clark, Cools et al. 2004; Budhani, Richell et al. 2006). Also, children with CD/ODD and psychopathic traits showed increased vmPFC BOLD activation during punished reversal errors, which may provide the neural basis for deleterious decision-making in antisocial populations (Finger, Marsh et al. 2008). Moreover, this may underpin an inability to respond appropriately to negative reinforcement in order to self regulate behaviour (Rolls, Hornak et al. 1994), or to an increased preference for risk.

2.1.2.3 Summary

Together these prior studies highlight anatomical and functional differences between antisocial and typically developing children in the PFC, a region that is important for the regulation of emotion and social behaviour. However, it is increasingly apparent that brain regions do not function in isolation, and that childhood antisocial phenotypes are likely to arise from the combination of activity and function occurring *between* interconnected brain regions. For example, one fMRI study found activations in different brain regions during a cognitive task in children with CD compared to boys with ADHD and healthy controls. Specifically, during a rewarded sustained attention task boys with CD showed reduced right orbitofrontal cortex activation, whereas during the unrewarded condition they showed reduced activation in the cerebellum, insula and several paralimbic regions (anterior cingulate cortex, hippocampus, and parahippacampal gyrus). The authors interpreted these results as an indication that difficulties in CD children may stem from dysfunction within the motivational *network* between the orbitofrontal and paralimbic brain regions (Rubia, Smith et al. 2009). Support for this hypothesis comes from, firstly, the observation that within many neuroimaging studies of antisocial/CU children (outlined above) deficits are seen in both the PFC and temporo-limbic/emotion processing regions, highlighting the overlapping nature of these deficits within these populations. Importantly, however, a number of studies have examined the 'connectivity' between these two regions and found evidence that this is reduced in antisocial populations. This evidence is discussed here.

2.1.3 Regional ‘connectivity’ in antisocial behaviour

While previous neuroimaging studies have examined discrete brain regions in relation to psychopathology, it is clear that brain regions do not function in isolation. Thus, neuroimaging research is now moving towards a more integrated ‘network’ based approach. Two key networks are implicated in antisocial behaviour research, and these comprise structures within the limbic system and the limbic-prefrontal network.

2.1.3.1 The limbic system

The limbic system includes the amygdala, hippocampus, fornix, mamillary bodies, anterior thalamic nucleus, and the cingulate gyrus (MacLean 1992). The limbic system is highly interconnected with other regions of the brain, including the midbrain, and brainstem, and the prefrontal cortex (Mark, Daniels et al. 1995).

This network underlies functions pertaining to emotion, memory, feeding, sexual and reproductive behaviour, aggression, and sociability (MacLean 1992). Abnormalities in limbic structures are associated with numerous neuropsychiatric disorders; these include anxiety disorders (Rauch, Shin et al. 2006), schizophrenia (Shenton, Dickey et al. 2001), and bipolar disorder (Brambilla, Hatch et al. 2008). Of relevance to this thesis, as discussed above, abnormalities within limbic structures (particularly the amygdala and

hippocampus) are also associated with CD (Sterzer, Stadler et al. 2007; Huebner, Vloet et al. 2008), and adult antisocial behaviour (Yang, Raine et al. 2009; Boccardi, Ganzola et al. 2010).

Figure 2.3: The limbic system

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The limbic system (Health Communities Inc; 1998-2010)

2.1.3.2 The limbic-prefrontal network

The limbic-prefrontal (also referred to as fronto-limbic) circuit comprises the amygdala, anterior cingulate, and PFC, and it is involved in social behaviour

and emotional regulation (Davidson, Putnam et al. 2000; Figure 2.4). Animal studies show that most afferent fibres arriving at the amygdala do so from the mPFC (Carmichael and Price 1995; Ghashghaei and Barbas 2002); and that this circuit is completed by projecting efferent fibres into the mPFC (Carmichael and Price 1995). These afferent and efferent fibres form the major white matter tract connecting the amygdala and PFC: the uncinate fasciculus (UF).

Figure 2.4: The limbic-prefrontal network

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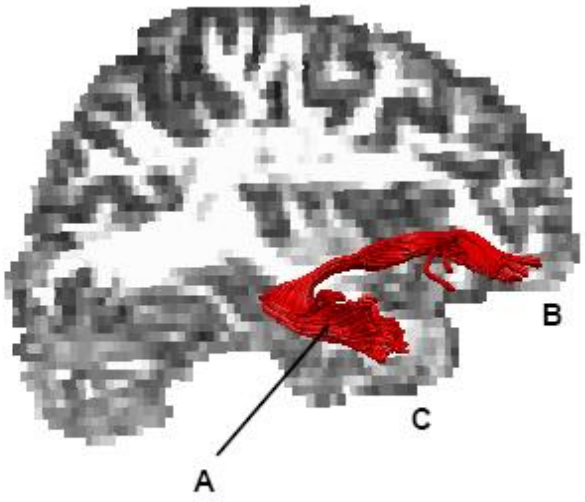
Figure 2.4: Diagram of limbic-prefrontal network (Heimer 2003)

The UF is a short, hook-shaped, limbic tract that connects the grey matter of the vmPFC with the amygdala and hippocampus within the temporal lobe (Catani, Howard et al. 2002; Craig, Catani et al. 2009 - see Figure 2.5).

Figure 2.5: The uncinate fasciculus



The uncinate fasciculus (A) connects the ventral prefrontal cortex (B) with the anterior temporal lobe (C)

The UF has the slowest maturation of all white matter tracts. It continues developing well into mid-adulthood and this renders it susceptible to developmental abnormality and related behavioural and neuropsychiatric disorders (Lebel, Walker et al. 2008). For instance, microstructural abnormality of this tract has been reported in emotionally deprived children, and young adults with ASPD, psychopathy, and schizophrenia (Craig, Catani et al. 2009; Kawashima, Nakamura et al. 2009; Motzkin, Newman et al. 2011; Sundram, Deeley et al. 2012).

2.1.3.3 Evidence for network dysfunction in antisocial behaviour

The role of limbic/limbic-prefrontal networks in antisocial behaviour disorders has been elucidated by advances in MR techniques. Two particularly

informative methods for investigating neural circuits are functional connectivity analysis, and DT-MRI. Functional connectivity analysis uses fMRI to detect temporal relationships between the activations of different brain regions during the performance of particular tasks. Temporally connected activations denote regions that are functionally interconnected during the particular mental tasks under investigation. In contrast, DT-MRI investigates the integrity of anatomical connections between different brain regions (please see Chapter 4 for full description of DT-MRI). Evidence for network deficits in antisocial behaviour from these types of study is presented below.

2.1.3.3.1 Functional 'connectivity'

Reduced fronto-limbic functional 'connectivity' has been found in children with conduct problems as compared to healthy controls (Marsh, Finger et al. 2008a; Decety, Michalska et al. 2009). In one study boys were shown animated films depicting individuals experiencing pain through injury. Compared to healthy boys, those with aggressive CD showed significantly greater BOLD activations in the amygdala, striatum, and temporal pole, despite showing normal activations in regions related to the pain matrix (e.g. insula, anterior cingulate cortex) when observing others being injured. Moreover, when watching acts of instrumental aggression (when the injuries had been inflicted deliberately by another person), CD boys showed significantly increased activations in medial prefrontal and lateral orbitofrontal cortices, alongside temporo-parietal regions, in comparison to healthy boys. When examining functional connectivity between the amygdala and prefrontal lobe, the authors found reduced 'coupling'

between these areas in the CD group only. This was taken to suggest abnormal neural responding to the pain of others may arise through this aberrant functional connectivity (Decety, Michalska et al. 2009).

A further functional connectivity study reported abnormal BOLD activations in children with elevated levels of CU traits and CD/ODD during an emotion processing paradigm (Marsh, Finger et al. 2008a; outlined above). These children showed significantly reduced amygdala activation to fearful expressions compared to healthy children and to those with ADHD. However, there were no significant between group differences in activation to angry or neutral faces, suggesting that these children have a specific deficit in fear reactivity. Importantly, functional connectivity analysis, examining the correlations between individual activations of the amygdala and vmPFC, found significantly reduced functional connectivity between these regions only in the children with CU traits (Marsh, Finger et al. 2008a).

Taken together the evidence from these two studies, and other reports of dysfunction in the orbitofrontal-paralimbic network (discussed above; Rubia, Smith et al. 2009) suggests that children with antisocial behaviour disorders have abnormal functional connectivity between frontal and limbic regions. However, it is not clear whether this abnormality is underpinned by deficits in the neural substrate connecting those regions (i.e. in the white matter tracts). That this may be the case is suggested by studies of antisocial behaviour in adults (Craig, Catani et al. 2009; Motzkin, Newman et al. 2011; Sundram, Deeley et al. 2011).

2.1.3.3.2 White matter microstructural integrity

Anatomical abnormality of fronto-limbic 'connectivity' has been demonstrated in adults with psychopathic antisocial behaviour using DT-MRI (Craig, Catani et al. 2009; Motzkin, Newman et al. 2011; Sundram, Deeley et al. 2011). Significantly lower fractional anisotropy (FA) – a proxy measure of tract integrity (see Chapter 4 for fuller description) – was reported in the right UF of psychopaths as compared to healthy controls (Craig, Catani et al. 2009; Motzkin, Newman et al. 2011; Sundram, Deeley et al. 2011). Furthermore, left UF FA showed a significant inverse correlation with Factor 2 (antisocial behaviour) PCL score in the psychopathy group (Craig, Catani et al. 2009). These findings provided the first evidence for a fronto-limbic white matter basis to the deficits seen in antisocial adults.

A further DT-MRI study examined adults with ASPD, and reported reduced FA of several regions compared to healthy controls. These included the right UF, internal capsule, and bilateral corpus callosum. Further, these reductions were inversely related to PCL-R psychopathy score (Sundram, Deeley et al. 2011). This study corroborates evidence for white matter abnormality in adult antisocial behaviour disorders.

In addition to these DT-MRI studies finding white matter abnormality in antisocial adults, a single voxel-based morphometry (VBM) sMRI study also reported white matter deficits in antisocial adults. Abnormalities of the corpus callosum were found in psychopathic males, including decreased thickness and

increased interhemispheric ‘connectivity’, relative to matched controls (Raine, Lencz et al. 2003). This supports the findings of Sundram et al (2011), and may suggest an association between corpus callosum abnormality and the reduced cerebral lateralization that has also been observed in psychopathic samples (Raine, O'Brien et al. 1990; Kiehl, Hare et al. 1999).

Therefore, there is initial evidence for white matter abnormality in adults with antisocial behaviour disorders. However, it is unknown whether the differences that were found are a cause or consequence of having been antisocial for many years. Also the results of these studies may be confounded due to the high levels of co-morbid substance abuse commonly found in such adult samples. That is, white matter integrity may be modulated as a consequence of having a generally dysfunctional lifestyle, or prolonged substance abuse, in these individuals rather than being a correlate of antisocial personality or psychopathic temperament *per se* (Versace, Almeida et al. 2008). An essential question, therefore, is whether antisocial children have differences in white matter tracts that connect brain systems implicated in the disorder. To date, no study has reported on microstructural integrity of white matter tracts in CD or CU children as measured using DT-MRI, although a recent VBM based MRI study reported reduced white matter concentration in frontal and temporo-limbic regions in boys with high, versus low, levels of CU traits (De Brito, McCrory et al. 2011). This supports the suggestion that that white matter abnormality may be present in antisocial children.

2.1.3.3.3 White matter and development

White matter consists of bundles of axons that are ensheathed by myelin, a lipid layer that insulates them and improves the speed and efficiency of neurotransmission; it is one of the determinants of tract integrity, and contributes towards measures of FA (Beaulieu 2009). Myelination of axons begins during the last two months of gestation, extending to first reach the frontal lobes at around 8 or 9 months postnatally (Marsh, Gerber et al. 2008; Deoni, Mercure et al. 2011). This process continues throughout infancy and childhood, with the final areas to myelinate being the anterior temporal and frontal lobes in late adolescence (Bartzokis 2005). That these are the same two brain regions implicated in childhood antisocial behaviour (described above) suggests that the extended maturation period of white matter in these brain regions may be relevant to these disorders (Lebel, Walker et al. 2008).

There is evidence that the development of white matter may be affected by prenatal developmental insult. Animal studies have reported a significant association between prenatal maternal stress and abnormality of brain 'connectivity' (Wiggins and Gottesfeld 1986; Dunlop, Archer et al. 1997; Ulupinar and Yucel 2005). First, slower rates of myelination are seen in the optic nerve of foetal sheep as a result of corticosteroid ingestion by their mothers (Dunlop, Archer et al. 1997). Second, disrupted neural 'connectivity' has been reported in rats, with decreased numbers of synapses present in the cerebellum of prenatally stressed offspring (Ulupinar and Yucel 2005). Finally,

rat offspring also showed early hypermyelination of motor areas prenatal stress, which then stabilised by day 40 (Wiggins and Gottesfeld 1986).

Hence, animal studies suggest that abnormal brain 'connectivity' can arise from a stressful antenatal environment; and that this may be partially mediated by increased levels of stress hormones such as cortisol. However, it is currently unclear whether white matter integrity and/or myelin content differs between antisocial and typically developing children, or whether antenatal stress affects white matter development in human offspring. Nevertheless, there is currently evidence from animal and human research that several other aspects of infant development are associated with antenatal maternal stress and/or anxiety – namely the HPA-axis, cognition and behaviour, and grey matter structure; this evidence is discussed in Chapter 3.

2.1.4 Summary

In summary, children with CD and high levels of CU traits show a pattern of structural and functional brain abnormality; and this is predominantly within prefrontal and limbic regions. That these deficits frequently co-occur suggests that CD is associated with developmental abnormalities in the limbic-prefrontal network. There is also evidence that abnormal neural 'connectivity' may arise from prenatal stress. Modern neuroimaging techniques now facilitate the examination of brain circuits in the investigation of psychopathology; and so this

thesis uses DT-MRI to determine whether ‘connectivity’ deficits are associated with adolescent CD and prenatal stress.

3: Prenatal influences on neurodevelopment and behaviour

There is a growing body of evidence that exposure to maternal negative mood, particularly stress and anxiety, in the prenatal period, can contribute to difficult temperament and behaviour problems in children. Behavioural problems in life-course persistent CD can be evident in infants from even before the age of 3 years of age (Moffitt, Caspi et al. 1996). For example, one longitudinal study found that infant temperament traits (e.g. fussiness, activity levels) up to age 1 were predictive of mother-rated conduct problems at age 4-13 (Lahey, Van Hulle et al. 2008). That temperament evident at such an early developmental stage can predict long lasting psychopathology points to the potential relevance of very early infant development and to, perhaps, factors predating birth. This chapter discusses evidence for the influence of prenatal stress/anxiety on child behaviour, and then gives an overview of some of the mechanisms that may underlie this.

3.1 Stress in pregnancy

3.1.1 Effects of prenatal factors on behavioural development

The links between stress, negative mood states (e.g. anxiety and depression), and the major stress hormone, cortisol, during pregnancy and adverse behavioural outcomes in offspring, are increasingly being recognised. Specifically, a number of large longitudinal studies have reported significant associations between prenatal stress and/or anxiety, and offspring behaviour

and conduct problems (O'Connor, Heron et al. 2002a; Van den Bergh and Marcoen 2004; Gutteling, de Weerth et al. 2005b; Van den Bergh, Mennes et al. 2005; Barker and Maughan 2009; Rice, Harold et al. 2010).

A number of studies have associated antenatal mood/cortisol with very early infant temperament and behavioural problems (see Appendix 1). Temperament is defined as the profile of emotional, attentional and motor reactivity that an infant shows, and this varies considerably between individuals and contributes to personality development (Rothbart 2007). As many of these characteristics are evident from very early in infancy, temperament is believed to have a considerable biological basis that can be influenced by the antenatal environment (Pesonen, Raikkonen et al. 2006). Prenatal stress is associated with raised maternal cortisol levels (de Weerth, van Hees et al. 2003), and with early difficult temperament, problem behaviour (Huizink, de Medina et al. 2002; Gutteling, de Weerth et al. 2005b), and anxiety (Austin, Leader et al. 2005) in offspring. Further, maternal stress has been linked to increased fear reactivity in infants (Bergman, Sarkar et al. 2007). Similarly, exposure to antenatal maternal depression and to elevated maternal cortisol levels are associated with negative reactivity in infants (Davis, Glynn et al. 2007). Both fearfulness and negative reactivity in infants are thought to reflect systems underlying the development of emotional control. Negative reactivity refers to the amount of negative emotional expression or 'fussiness' that is displayed by the infant. This is thought to pose a risk for externalising behaviour as it may reflect emotional dysregulation (Bates, Freeland et al. 1979; Snyder, Reid et al. 2003),

which implicates the involvement of limbic brain structures, such as the amygdala.

Studies of older children have also found prenatal stress/anxiety to be related to externalising behaviour, conduct problems and emotional problems (O'Connor, Heron et al. 2002b; O'Connor, Heron et al. 2003; Barker and Maughan 2009; Rice, Harold et al. 2010). In addition, prenatal stress/anxiety is associated with ADHD (Van den Bergh and Marcoen 2004), and impulsivity (Van den Bergh, Mennes et al. 2005). Finally, one recent study has reported an association between maternal antenatal depression and teenage antisocial behaviour (Hay, Pawlby et al. 2010). These findings indicate that maternal emotional factors during pregnancy may contribute to long term behavioural outcomes in children. Hence, it has been suggested that "the attributable load in behavioral (sic) problems due to antenatal anxiety is in the order of 15%" (Talge, Neal et al. 2007). While stress and anxiety are not considered equivalent, it is accepted that stress gives rise to the negative mood states of anxiety and depression (Cohen, Janicki-Deverts et al. 2007), which are also linked to offspring behavioural outcomes.

A further line of evidence supports the association between stress and offspring behaviour. A number of studies have linked maternal smoking during pregnancy to serious offspring antisocial behaviour (Brennan, Grekin et al. 1999; Rasanen, Hakko et al. 1999; Wakschlag, Pickett et al. 2002) and persistent offending (Brennan, Grekin et al. 1999)). Prenatal tobacco exposure is also associated with Factor 1 (affective/interpersonal) PCL scores in

adolescents (Burke, Loeber et al. 2007), and to psychopathic traits in teenagers with ADHD (Fowler, Langley et al. 2009). While the mechanisms by which these effects arise are unclear, it is known that nicotine leads to increases in both stress (Parrott 1999) and cortisol (Hill and Wynder 1974; Wilkins, Carlson et al. 1982). In addition, stress and smoking during pregnancy are each independently linked to the same adverse birth outcomes (lower birth weight, smaller head circumference, and lower gestational age) (Lou, Hansen et al. 2008). Thus, maternal smoking may give rise to antisocial behaviour/psychopathic traits as a result of effects of elevated maternal stress/cortisol on the foetal brain.

3.1.2 Effects of prenatal factors on cognitive development

As well as its effects on behaviour, antenatal stress/cortisol has also been shown to influence offspring cognitive and behavioural development. Animal studies indicate that gestational stress/anxiety impairs spatial learning (Yaka et al, 2007), and causes attention deficits (Schneider et al, 2002) in offspring. Human studies also reveal associations between prenatal stress, anxiety, or depression and offspring cognition; for example, greater stressful life events experienced during pregnancy was associated with reduced mental development of infants at ~17 months (Bergman, Sarkar et al. 2007). Also, reduced IQ was found in teenagers of prenatally anxious mothers (Van den Bergh, Mennes et al. 2005). Further, pregnancy-specific anxiety is associated with reduced inhibitory control and working memory deficit in 6 to 9 year olds

(Buss, Davies et al. 2011). These neurocognitive abnormalities suggest that neural systems underlying these processes may be undergoing aberrant development in response to prenatal events. Further, this evidence indicates that the aforementioned behavioural effects may also arise from abnormal development of these brain regions.

3.2 Putative mechanisms linking prenatal stress and offspring behaviour

The precise mechanism through which maternal antenatal stress and behavioural development may be associated is not currently clear. However, human and animal research shows that stress, anxiety, and cortisol can influence both the neuroendocrine system and the brain of developing foetuses; this evidence is outlined below.

3.2.1 Prenatal stress, cortisol and the hypothalamic-pituitary-adrenal axis

Maternal antenatal mood and cortisol may influence behavioural development through disrupting the development of the foetal neuroendocrine system, namely the HPA-axis (please refer to Chapter 2 for full description of the HPA-axis; Sullivan, Hawes et al. 2008). The HPA-axis is fundamental for enabling responding to stress (Phillips and Jones 2006). For example, both animal and human offspring exposed to prenatal stress show increased resting and stress-

response glucocorticoid levels (Fride, Dan et al. 1986; Takahashi, Haglin et al. 1992; Weinstock, Matlina et al. 1992; Vallee, Mayo et al. 1996; Weinstock, Poltyrev et al. 1998; Gutteling, de Weerth et al. 2004; Gutteling, de Weerth et al. 2005a; O'Connor, Ben-Shlomo et al. 2005).

Hyper-reactivity of the stress system may render individuals more sensitive to environmental threat and thus contribute to aggressive conduct (Glover 2011). Support for this suggestion comes from studies reporting that boys with CD show elevated cortisol levels as compared to healthy controls, and that cortisol levels are greatest in those showing high rates of reactive aggression (Van Bokhoven, Van Goozen et al. 2005). In contrast, however, others have found cortisol *hypo*reactivity in CD (McBurnett and Lahey 1994; van Goozen, Matthys et al. 1998; Fairchild, van Goozen et al. 2008b), and in those with high levels of CU traits (Loney, Butler et al. 2006). Further, serum cortisol levels of young violent offenders are inversely related to severity of PCL-R Factor 1 psychopathy scores (Holi, Auvinen-Lintunen et al. 2006). Together these studies suggest that there may be an association between HPA-axis *dysfunction* (as opposed to simply hyper or hypo reactivity) and CD with and without CU/psychopathic traits. Also, taken together, this evidence illustrates how elevations in antenatal glucocorticoids, which can arise from stress exposure, can lead to long lasting effects on the infant stress response and potentially give rise to conduct problems.

3.2.2 Prenatal stress, cortisol and neuroanatomical development

The link between prenatal adversity and cognitive and behavioural outcomes in children is not only linked to HPA-axis dysfunction, but also to aberrant development of the neural regions associated with these cognitive functions and behaviours. In particular, exposure to prenatal stressors is associated with aberrant development of prefrontal and limbic brain regions. For instance, prenatally stressed rats show higher cell counts (Salm, Pavelko et al. 2004) and altered development (Kraszpulski, Dickerson et al. 2006) of amygdaloid nuclei, with the latter correlating with increased fearfulness in offspring. Conversely, hippocampal volume and weight is reduced (Szuran, Zimmermann et al. 1994; Uno, Eisele et al. 1994; Coe, Kramer et al. 2003), alongside elevated cortisol output (Coe et al, 2003), in offspring of acutely stressed non-human primates during pregnancy. A recent study of prenatally stressed rats found reduced dendritic complexity and spine density of hippocampal granule cells. This was reported along with the down-regulation of hippocampal mineralocorticoid receptors, which the authors suggest may result from elevated maternal transfer of corticosterone during gestation (Tamura, Sajo et al. 2011). Other studies also found reduced hippocampal glucocorticoid receptor density in prenatally stressed rat offspring (Henry, Kabbaj et al. 1994; Szuran, Pliska et al. 2000). Finally, abnormal white matter development has also been found to result from elevated cortisol exposure during pregnancy. For example, restraint stress administered to rats during gestation resulted in early hypermyelination in the brains of pups (Wiggins and Gottesfeld 1986). Also, neural 'connectivity' was reduced in a study of prenatally stressed rats, who showed a reduced number

of cerebellar synapses (Ulupinar and Yucel 2005). Further, the prenatal administration of betamethasone results in reduced myelination, axon diameter, and myelin thickness in sheep (Huang, Harper et al. 2001). Myelination contributes to white matter integrity, which influences brain connectivity (Beaulieu 2009). Interconnection of brain regions is important for the generation of complex emotional and behavioural functions such as those involved in the generation of conduct problems (see Chapter 2 for a review of this evidence). It is for this reason that the investigation of white matter neuroanatomy in prenatally stressed populations is vital.

Relationships between prenatal stress/anxiety and neurodevelopment have also been observed in humans. One study identified reduced head circumference in offspring of prenatally stressed mothers (Lou, Hansen et al. 2008), although a further study failed to replicate this (Obel, Hedegaard et al. 2003). A more recent study used MRI and reported brain structural deficits in children aged between 6 and 9, whose mothers experienced anxiety during mid-gestation (Buss, Davis et al. 2010). Reduced grey matter density in several regions - including prefrontal and temporal cortices - was significantly associated with prenatal anxiety levels. This is the only human neuroimaging study linking prenatal mood to structural abnormalities that may potentially underlie the adverse behavioural, cognitive and emotional outcomes seen in exposed offspring. Importantly, the brain regions in which structural anatomy was associated with prenatal anxiety in this study (i.e. PFC and temporal lobe) - as well as the animal studies outlined above – overlap with those found in children with CD/CU traits (reviewed in Chapter 2).

However, despite the overlap between brain regions implicated in antisocial behaviour disorders and prenatal stress/anxiety, three questions remain unanswered. First, it is not known whether there is abnormal white matter tract integrity in children with CD/psychopathic traits. Second, it is unclear whether antenatal maternal stressful life events are associated with differences in white matter tract development of their offspring and, third, no study has investigated whether – as suggested by animal studies - deficits in human white matter tract development are related to *in utero* cortisol concentration. Part of this thesis therefore addresses these questions.

In summary, neuroimaging data from studies of children and adolescents with CD with/without CU traits have identified a number of brain structural and functional deficits; and these predominantly (but not exclusively) affect the prefrontal and limbic regions. However, to date no studies of CD have specifically examined the white matter pathways between these regions using DT-MRI (i.e. the limbic-prefrontal network). This thesis therefore aims to assess white matter microstructural integrity in children with CD and CU traits, and examines the extent to which it is associated with variation in the behavioural phenotype (please see Chapters 5 and 6). Finally, the thesis explores a putative mechanism by which conduct problems and/or white matter abnormality may arise, namely via maternal antenatal stress and/or elevated *in utero* cortisol. Thus, Chapter 7 of the thesis presents a preliminary investigation into the effects of these factors on white matter, also using DT-MRI.

3.3 Aims of the thesis

The aims of the three studies that constitute this thesis are as follows:

3.3.1 Study 1

3.3.1.1 Investigation 1

1) To investigate the hypothesis that abnormalities in limbic-prefrontal white matter connectivity affecting the uncinate fasciculus reported in antisocial adults also exist in boys with Conduct disorder in comparison to healthy boys.

3.3.1.2 Investigation 2

2) To perform the first whole-brain investigation of white matter to identify additional regions where differences in white matter integrity are evident in these populations.

3.3.2 Study 2

3.3.2.1 Investigation 3

3) To explore whether antenatal maternal stressful life events and/or *in utero* cortisol are associated with developmental differences in these white matter tracts.

The following chapter details the materials and methods employed in order to conduct the three investigations that comprise this thesis.

4: Materials and methods

4.1 Overview

The two studies that constitute this thesis both examined white matter tract integrity using Diffusion Tensor Magnetic Resonance Imaging (DT-MRI); however, two different cohorts were examined. In Study 1 I examined white matter correlates of childhood antisocial behaviour. I recruited and compared a group of adolescent males with Conduct disorder with a group of typically developing controls The results of two types of white matter analysis - tractography, and Tract-Based Spatial Statistics (TBSS) - from this sample are reported in Chapters 5 and 6 of the thesis, respectively. In Study 2 I employed DT-MRI tractography in order to explore white matter development in relation to maternal antenatal stress/cortisol. I examined a group of children aged between 6 and 9 years old who, while typically developing, were part of an existing research cohort. These children were borne of mothers who had been recruited during pregnancy, and for whom measures of prenatal stress and *in utero* cortisol concentration were known; the results are reported in Chapter 7.

The current chapter will outline the materials and methods employed in the recruitment and assessment of each study sample in turn. Details of the steps taken for all neuroimaging data analysis are explained within the methods for Study 1, as these same steps were duplicated in Study 2.

4.2 Study 1

This study compared white matter microstructural integrity using both tract based and whole brain DT-MRI analysis of adolescent boys with Conduct disorder and healthy control males who did not differ significantly in age, IQ, handedness, and other potential confounding variables (e.g. ethnicity, history of substance use, and socio-economic status). The following sections describe the process that I underwent in order to operationalise these aims.

4.3 Ethical issues and Ethical approval

Ethical approval for this study had been granted in 2007 by the Joint South London and Maudsley Research Ethics Committee following the submission of an addendum to an existing adult study (243/00; see Appendix 2). I needed to compile and submit three amendments to the study ethics in order to bring the ethics in line with my research aims. These were: (1) a request for the addition of control participant recruitment; (2) an amendment to the recruitment flyer; and (3) several minor amendments to the study protocol (see Appendix 3).

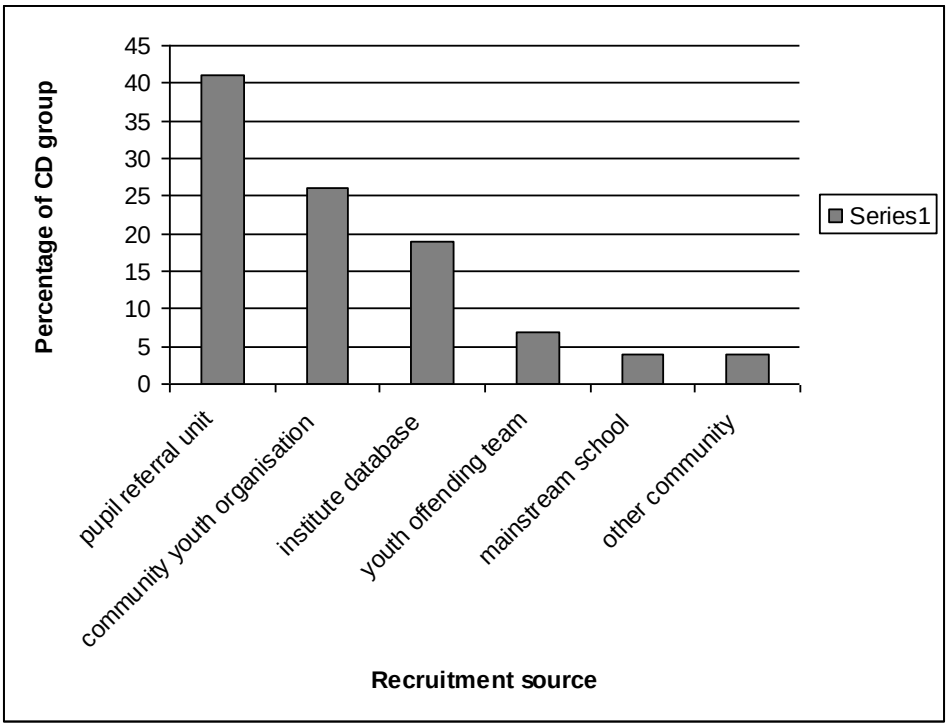
4.4 Participants

4.4.1 Recruitment

4.4.1.1 Conduct disorder group

I recruited twenty-eight right handed males - aged between 12 and 19 years - through several sources: (i) an Institute of Psychiatry database of adolescents with conduct problems; (ii) three London-based Youth Offending Teams (Haringey, Lewisham, and Southwark); (iii) five Pupil Referral Units (PRU; facilities that provide education to children who cannot attend mainstream schools, for example, following school exclusion); these were Cavendish School, Bridge Academy, Park Campus, Phil Edwards School, and Southwark Inclusive Learning Service; (iv) four youth organisations (Brimmington Youth Club, Catch-22, Ebury Bridge Youth Club, and Reprezent); (v) two other educational institutions (The Bosco Centre and Pimlico Academy); and (vi) other community recruitment, acquaintances recommended by study participants (see Figure 4.1).

Figure 4.1: Sources of Conduct disorder group recruitment



CD – Conduct disorder

I approached Pupil Referral Units, schools and youth organisations by telephone, followed by a meeting with the head teacher; after this, schools assisted me by sending a Patient Information Sheet (PIS; see Appendix 4a) to parents of adolescent male pupils with a recent history of conduct problems. Parents/guardians of interested boys were invited to make contact with me directly by telephone or via the study-specific email address I had created. During initial contact I imparted information about the study to parents/guardians, addressed queries, and conducted a brief telephone screening to ensure exclusion criteria were not met by the children. Exclusion

criteria were: left handedness, being below 12 or above 19 years old, any psychiatric history (excluding Oppositional Defiant disorder (ODD), Conduct disorder (CD) or Attention Deficit Hyperactivity Disorder (ADHD)), any history of neurological injury, and physical health contraindications for MRI scanning. Finally, I asked parents/guardians about their son's behaviour within the last year and throughout childhood, using the four 'Conduct Problem' items on the parent version of the Strengths and Difficulties Questionnaire (SDQ; Goodman, 1999; see Questionnaires) and the screening items in the Kiddie Schedule for affective Disorders and Schizophrenia – Present and Lifetime (K-SADS-PL; Kaufman, Birhamer et al. 1997) interview. Where scores fell within the 'abnormal' range (exceeding the recommended parent version cut-off of 4) on the SDQ, and where more than one current K-SADS-PL item was met, an appointment was arranged. Verbal consent was taken from parents to be sent parent and child questionnaire packs to fill in prior to their test date (see Postal Questionnaires below). Three children, for whom conduct problems were not evident through SDQ and K-SADS-PL responses, were included in the healthy control group.

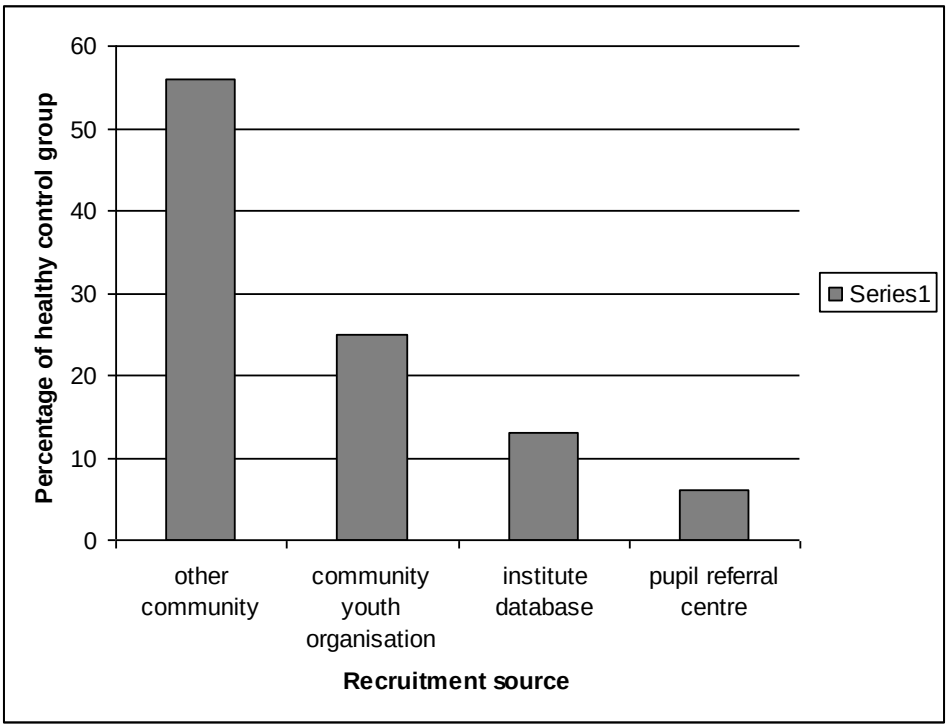
In addition to schools, boys were also recruited from Youth Offending Teams (YOT). I initially approached London YOT teams by email, followed by a telephone call to address any queries. During these calls, I provided staff members with details of inclusion criteria: a history of conduct problems and offending behaviour within the last 12 months, right handedness, and also a number of personality traits characteristic of CU temperament. Meetings were held at two YOTs (Lewisham and Lambeth) whereas other teams referred

individuals directly following telephone and email contact (Haringey and Southwark). Key workers approached suitable and interested boys, and then provided contact details for the individual (if aged 16 or above), or their parent/guardian (if below 16). Finally, I made telephone contact, and individuals were screened and recruited as outlined above.

4.4.1.2 Healthy control group

In addition to the CD group I also recruited a healthy control group, consisting of sixteen right handed males aged between 12 and 19 years old. Boys were recruited from the general public through placement of a recruitment flyer (see Appendix 5) in several locations: (i) on the Gumtree website; (ii) on the notice board of several south London branches of the 'Connexions' youth advisory service and through several youth organisations (Brimmington Youth Club, Ebury Bridge Youth Club, and Reprezent) within the same geographical area from which the CD group was recruited; (iii) an Institute of Psychiatry database of healthy controls; and (iv) PRUs (see Figure 4.2). Parents/guardians contacted me directly after they or their son had seen the recruitment flyer. As with the CD group, interested boys were appointed after a brief telephone screening to exclude left-handedness, history of conduct problems, any psychiatric history (excluding ADHD), any neurological history, and contraindications for MRI.

Figure 4.2: Sources of healthy control group recruitment



4.4.2 MRI Safety

In order to screen for MRI compatibility, all potential participants (via their parents if below 16 years old) were asked whether they had any metallic objects in their body (e.g. as a result of accident or injury involving penetration by metal items/shrapnel), or had ever undergone surgery involving insertion of metal clips (see Appendix 6 for MRI Safety Form). Where boys had undergone surgery, consent was taken from parent and child to obtain surgical notes. These were requested by fax from the 'Access to Medical Records' department of the relevant hospital (see Appendix 7). Notes were received within two to

three weeks by post, and were checked by radiographers at the Centre for Neuroimaging Science (CNS), Institute of Psychiatry. Where procedures were deemed safe for scanning, details were recorded onto the CNS database, after which participants' surgical notes were destroyed. All study participants satisfied MRI safety requirements.

4.5 Materials

4.5.1 Postal Questionnaires

Having established MRI compatibility, verbal consent was taken from participants and parents to be sent the following questionnaires by post:

Table 4.1: Postal questionnaires sent prior to assessment date

	Parent report	Child self-report
Strengths and Difficulties Questionnaire	√	√
Antisocial Process Screening Device	√	√
Edinburgh Handedness Inventory	-	√
Substance use scale	-	√

Individuals were asked to complete questionnaires, seal them into the stamped addressed envelope provided, and return to me by post along with a signed consent form (Appendix 4b). Sending questionnaires by post served to reduce testing time on the assessment day; questionnaires were labelled with study numbers in order to anonymise them. These measures are described below, alongside other instruments used in the study.

4.6 Measures

4.6.1 Independent variables

4.6.1.1 Conduct disorder

As noted above, two measures were used to characterise the Conduct disorder group, these were the SDQ and the K-SADS-PL (see Appendices 8 & 9).

4.6.1.1.1 Strengths & Difficulties Questionnaire (SDQ; Goodman 1999) – Self-/Parent report

The SDQ is a 25-item screening measure that assesses behavioural attributes falling within five categories: emotional symptoms, conduct problems, hyperactivity/inattention, peer relationship problems, and prosocial behaviour. Items from each of these subscales yield a possible total of 10 points, with

different recommended cut-offs into the 'abnormal' range on self- (5 or above) and parent report (4 or above) versions of the questionnaire. The conduct problems subscale was used to screen for the presence of conduct problems. Where either informant's score fell within the 'abnormal' range complete CD and ODD sections of the K-SADS-PL (see below) interview were administered, in order to make a research diagnosis of current Conduct disorder. The SDQ has high predictive and discriminative validity in the detection of conduct problems, hyperactivity and other child psychiatric problems (Goodman, Ford et al. 2000), and shows good cross-cultural sensitivity (Goodman, Renfrew et al. 2000).

The self-report and parent report versions of the SDQ are recommended for use in 11-17 and 4-16 year olds, respectively, but show utility in older samples (Svedin and Priebe 2008). That some participants were aged above 16/17 in the current study was not thought likely to significantly impact on the utility of this measure, only in the fact that some items were worded for younger individuals. However, respondents were forewarned that this may be the case and instructed to consider the questions to themselves/their sons in a more age appropriate way if necessary (e.g. Item 4 'shares readily with other children (toys, treats, pencils, etc)' to be considered 'shares his things readily with others', etc). Furthermore, the SDQ was used only as a screener, whereas the K-SADS-PL interview was used to allocate participants to the CD group. However, despite not being used for group designation, both the conduct problems and total problems SDQ scores (i.e. the sum of scores from the first four subscales of the SDQ) were used as covariates within the subsequent analysis. Similarly, the hyperactivity/inattention score was used in analysis, to

ensure that groups did not significantly differ on these symptoms (see Table 4.3 below). Other researchers have used these subscales to explore between group differences in hyperactivity (e.g. Jones, Laurens et al. 2009).

4.6.1.1.2 Kiddie-SADS – Present and Lifetime (K-SADS-PL; Kaufman, Birhamer et al. 1997) – Child interview

This second measure of conduct problems used in the study was the child version of the SADS (Schedule for Affective Disorders and Schizophrenia) interview, the K-SADS-PL. This instrument consists of 17 semi-structured interview questions that are based on DSM (Diagnostic and Statistical Manual of Mental Disorders; III, IV-TR; 1980; American 2000) criteria for present or lifetime Conduct disorder in 6 to 18 year olds. The interview schedule contains a number of prompts for each item, upon which the interviewer can build in order to obtain adequate information upon which to score each item.

Each K-SADS-PL item may be given one of three scores: 0 - where no information is present; 1 - where the behaviour is not exhibited; 2 - where the behaviour has occurred but is below threshold; or 3 - where the threshold for the item is met. In this study, allocation to the CD group was based on a DSM-IV-TR diagnosis of current CD, and it was further noted whether CD had a childhood or adolescent onset. A diagnosis of current CD requires three of the listed behaviours to have been present within the previous year, with at least one occurring in the last six months. Furthermore, CD is classified as being ‘mild’, ‘moderate’, or ‘severe’ depending on the number and type of threshold

items met. For example, a child is classified as having severe CD when many items are met above threshold level and/or where these are behaviours that cause substantial harm to others, such as use of weapons and forced sexual activity. Mild CD is recorded where few items are met and these are not of a violent nature (e.g. truancy, shoplifting).

4.6.1.2 Psychopathic traits

Similar to the measurement of CD, psychopathic traits were assessed using two instruments. The first was the Antisocial Process Screening Device (APSD) that was used as a screening questionnaire, but was used as a covariate in later analysis, and the second – the Psychopathy Checklist – Youth Version (PCL-YV) interview - was used as a grouping measure (see Appendices 10 & 11 for these measures). These instruments are described here.

4.6.1.2.1 Antisocial Process Screening Device (APSD; Frick and Hare 2001) - Self-/parent report

The APSD is a twenty item behavioural measure, based on the Psychopathy Checklist Revised (see below), that is used to assess antisocial and psychopathic traits in children aged between either 6 and 13 years old (parent version), or between 13 and 18 (self-report version). Following personal communication with one of the questionnaire's authors (Paul Frick) it was concluded that because this instrument was to be used as a screening instrument, the fact that our cohort's age range exceeded the recommended

upper age limit for the parent version of this instrument was acceptable. Furthermore, the accuracy of self-report may, in fact, supersede that of informant report when children reach adolescence (Kamphaus and Frick 1996).

Both versions of the APSD contain three factors: callous-unemotional traits (6 items); narcissism (7 items); and impulsivity (5 items). Responses on the APSD are made using a three-point Likert scale, with a maximum possible score of 40. There is no established cut-off for this measure, and a variety of methods for selecting 'high' psychopathic traits populations have been suggested, including 90th percentile scores (Sharp, van Goozen et al. 2006) or arbitrary cut-offs such as 20 (Finger, Marsh et al. 2008), 25 (Budhani, Blair et al. 2005) or 27 (Blair, Mitchell et al. 2005). As the current study's participants were recruited from a predominantly community - rather than criminal justice – setting, and as the APSD was used as a screening instrument, a cut-off of 20 was used in accordance with previous work in similar population (Jones, Laurens et al. 2009). As with that study, I administered both self-report and parent report versions and used the highest informant rating for each item to obtain total APSD and CU subscale scores. Total score on the scales was used, first, to assign individuals to a 'high' or 'low' psychopathic traits subgroup within the CD group. This then determined who would be administered the PCL-YV interview (high scorers). Second, both total and CU trait score were used as dimensional covariates representing psychopathic and CU traits, respectively, within the entire cohort.

4.6.1.2.2 Psychopathy Checklist – Youth Version (PCL-YV; Forth, Kosson et al. 2003) - Child interview

The second measure of psychopathic traits I used in the study was the 20 item PCL-YV. This instrument is scored based on a semi-structured interview and, where available, collateral information. The PCL-YV measures psychopathic traits in adolescents aged between 12 and 18 year olds along two dimensions: (i) interpersonal/affective characteristics; and (ii) impulsivity/antisocial behaviour (see Table 4.2). Each item is scored on a 3-point Likert scale: 0 is given where the item does not apply to the individual, 1 when the item may apply, and 2 where the item definitely applies; the maximum possible score on the PCL-YV is 40. Similar to the APSD, no official 'cut-off' exists for this measure; so following previous researchers, scores above 20 identified boys with high levels of psychopathic traits (Jones et al, 2009).

Table 4.2: PCL-YV two-factor structure

Factor 1: Interpersonal/Affective	Factor 2: Behavioural/Antisocial
Impression management	Stimulation seeking
Grandiose sense of self worth	Parasitic orientation
Pathological lying	Lacks goals
Manipulation for personal gain	Impulsivity
Lack of remorse	Irresponsibility
Shallow affect	Poor anger control
Callous/lack of empathy	Early behaviour problems
Failure to accept responsibility	Serious criminal behaviour
	Criminal versatility
	Serious violations of conditional release

NB: Two further items do not load onto either factor: impersonal sexual behaviour and unstable interpersonal relationships.

In addition to scoring the PCL from the semi-structured interview, it is recommended that collateral information (e.g. file notes, police records, etc.) is used to score both the adult version (Psychopathy Checklist – Revised; PCL-R) and the PCL–YV. Although this study did not have ethical approval to obtain police files and other official records, information given by youths and parents on questionnaires and during telephone screening interviews, plus additional

details from teachers and youth club workers, was incorporated into the scoring of the PCL-YV. Previous studies have used the PCL-YV measure without collateral information and have found this to provide a valid assessment of psychopathic traits (Burke, Loeber et al. 2007). Prior to beginning the study I attended the Darkstone Research Group PCL workshop to receive training in the administration and scoring of the instrument.

Within this study, PCL-YV scores were used to categorically define a group of conduct disordered individuals who had high levels of psychopathic traits. Further, within this high psychopathic traits group scores were additionally used as a dimensional covariate to be examined against scanning data.

4.6.2 Potential confounding variables

I included a number of further measures in order to characterise the groups and ensure they did not differ significantly in terms of potential confounding variables; these included: handedness, substance use, ethnicity, IQ, ADHD symptoms, and socio-economic status.

4.6.2.1 Handedness

4.6.2.1.1 Edinburgh Handedness Inventory (Oldfield, 1971) - Self report

As DT-MRI studies demonstrate differences in white matter asymmetry between right and left handed individuals (Buchel, Raedler et al. 2004), I used the Edinburgh Handedness Inventory to screen out non-right handed participants to make the group homogenous. This tool lists ten daily activities (e.g. throwing a ball, writing, drawing) and individuals report whether they use their left, right, or both hands, to perform each activity. Scores are calculated based on the difference between the numbers of responses given for each hand, and taking into consideration those items for which both hands are used equally. Finally, it is possible to ascribe right, left or mixed handedness to respondents, with a score of -40 to -20 translating as dominant left handedness; -20 to +40 - mixed handedness; and +40 or above - dominant right handedness. Although verbal report of handedness was obtained at the screening stage of recruitment, this instrument was used to ensure participants were dominantly right handed. All study participants satisfied this criterion.

4.6.2.2 Substance use

4.6.2.2.1 Substance Use measure (adopted from the European school Survey Project on Alcohol and other Drugs (ESPAD); Hibell, Guttormsson et al. 2007) - Self report

Comorbid substance misuse is more common among children and adolescents with conduct disorder (e.g. Boyle and Offord 1991). The effect of different classes of recreational drugs on white matter is not fully understood, but cocaine dependent individuals have significant white matter differences as compared to healthy controls (Ma, Hasan et al. 2009), as do users of heroin and cannabis (Schlaepfer, Lancaster et al. 2005). Hence, I included a questionnaire to assess the possible confounding effects of drug use.

This questionnaire consisted of a subsection of the ESPAD survey that contains items to ascertain lifetime use of ten recreational drug classes: alcohol, cannabis, ecstasy, benzodiazepines, amphetamines, cocaine, heroin, inhalants, GHB (gamma-hydroxybutyric acid), and hallucinogens. Where respondents reported having ever used one or more of these, a further question asked how many times each substance had been used within the past year, offering the following response options: 1) 1-2 times; 2) 3-9 times; 3) 10-19 times; 4) 20+ times. In addition, for the three more commonly used substances – alcohol, cannabis, and ecstasy – a further question measured past month use using the same multiple choice options. Data were used to compare group substance use.

4.6.2.3 Ethnicity

Ethnicity was recorded in accordance with the South London and Maudsley NHS Trust ethnicity classification categories and codes (see Appendix 12). For the analysis, ethnicity was recoded into one of 3 categories: White Caucasian, Black/African-Caribbean, and Other ethnicity.

4.6.2.4 IQ

4.6.2.4.1 Wechsler Abbreviated Scale of Intelligence (WASI) - Child interview

The WASI (WASI; Wechsler 1999) assesses the intellectual ability of individuals aged 6 to 89 years based on a four subscales which can be used to obtain estimates of verbal, performance and full scale IQ (FSIQ). Depending on research requirements either two or all four sections can be used, and within the current study two subtests were administered to obtain FSIQ, based on the vocabulary and matrix reasoning tasks. The vocabulary subtest requires individuals to provide definitions of words from a list of increasingly complex items; responses for each word receive a score of 0, 1 or 2 points. Twelve to sixteen year old participants are administered 29 items, while older participants are administered 34 items. The matrix reasoning task requires children to examine a series of 29 pictures, each with a missing section that has been replaced by a blank box containing a question mark. In each case, individuals are asked to select one shape from five similar shapes, to best complete the

picture. Each correct selection scores one point, and items are administered until either 4 consecutive wrong answers are given, or 4 wrong answers are given on 5 consecutive items. I received full training in administering this instrument, and assessed all participants on each of the two subtests in order to ensure consistent scoring across all participants. Further, subsets were completed in the same order by all participants. As this measure was only included to ensure that the CD and comparison groups did not differ significantly in FSIQ, differences between verbal and performance IQ was not assessed.

Following testing, raw scores were converted into T-scores using normative tables provided with the instrument, and a FSIQ score for each participant was ascertained. These scores were used in Study 1 (Chapters 5 and 6) to ensure groups did not differ significantly in FSIQ, and was also used as a covariate in neuroimaging analyses. In Study 2 (Chapter 7) FSIQ was a dependent variable to, first, examine any relationship between overall intelligence and maternal prenatal stress or *in utero* cortisol concentration and, second, as a covariate within neuroimaging analyses.

4.6.2.5 ADHD

4.6.2.5.1 'Hyperactivity/inattentive' subscale of SDQ – Self/parent report

As discussed in Chapter 1, CD and ADHD are commonly comorbid (Abikoff and Klein 1992; Offord, Boyle et al. 1992), and individuals with ADHD have significant differences from controls in brain anatomy and function (Cherkasova and Hechtman 2009). Hence it is important to establish that any observed difference in brain structure and connectivity between CD and healthy controls is specific to CD and not to ADHD. Two measures were implemented within the current study in order to assess the prevalence of ADHD within the study population. First, at the initial telephone screening stage individuals (and/or their parent/guardian) were asked whether they had ever received a diagnosis of ADHD. Four individuals had previously received this diagnosis – two who met criteria for CD, and two healthy controls. Although this meant that each group contained an equal number of individuals with a prior diagnosis of ADHD, this was not sufficient to ensure the two groups equally represented individuals with hyperactivity and/or inattention symptoms as other boys may simply have not received a diagnosis from a medical practitioner. Therefore a second measure of ADHD was also obtained. Following the methods of other research groups (Jones, Laurens et al. 2009) the 'hyperactivity/inattentive' subscale of the Strengths and Difficulties Questionnaire (see above) was used in all individuals.

4.6.2.6 Socio-economic status

I used a parent report questionnaire that assessed parental employment, income and educational background. These three parameters are accepted indicators of social status in relation to health research (Liberatos, Link et al. 1988). In the present study an 'annual household income' question was used as a proxy measure of parental socio-economic status and responses were coded within the following categories: 1) less than £18,000; 2) 18,000-£25,000; 3) £25,000-£43,000; and 4) £43,000 or above. This questionnaire was developed by the Imperial College London Psychoneuroendocrinology team led by Professor Vivette Glover - who established the cohort in Study 2 (see Appendix 13) - and this instrument has been used in much published research (e.g. O'Donnell, Bugge Jensen et al. 2012).

4.6.3 Dependent variables

4.6.3.1 Diffusion Tensor Magnetic Resonance Imaging

The major outcome variables were the two white matter parameters acquired through a Diffusion tensor Magnetic Resonance Imaging (DT-MRI), namely fractional anisotropy (FA), and perpendicular diffusivity (D_{perp}). DT-MRI scanning took approximately 15 minutes per participant. Further details of neuroimaging and DT-MRI parameters are given in subsequent section: Neuroimaging.

4.7 Power calculation

Sample size was calculated using nQuery Advisor software (Elashoff 1996) and was estimated on the basis of a previously published study in adults which reported significant differences in fractional anisotropy, a DT-MRI parameter (see Neuroimaging, below), in a group of psychopathic antisocial males compared to healthy controls (Craig, Catani et al. 2009). A sample size of above 11 was recommended for 99% power and an effect size of 2.

4.8 Procedure

On the day of testing, participants were greeted at the Centre for Neuroimaging Sciences (CNS) building, Institute of Psychiatry, and taken through the patient information sheet, and any remaining questions were answered. Written, informed consent was taken from participants, and additionally from the accompanying parent/guardian where boys were aged below sixteen years old. Completed MRI safety forms (see Appendix 6) were checked and retained by the duty radiographer.

After gaining consent, participants were shown to a dummy MRI scanner in order to familiarise them with the scanner environment prior to their MRI session. Boys were asked to lie on the scanner table, where they were fitted with a pair of headphones, and finally fitted with the headcoil over their heads. The headcoil is fitted with two angled mirrors that enable participants to see out into the room whilst lying inside the scanner. A projector screen was fixed to the wall at one side of the room, upon which a short cartoon clip was shown via a film projector. In order to habituate participants to viewing the screen with their eyes focussed upwards, they were instructed to watch the cartoon for up to ten minutes through the angled mirrors above them while keeping their head still. This training session served to minimise anxiety among study participants, and to prevent potential withdrawals from the study due to participants feeling unprepared for MRI scanning.

Following the dummy scanner session participants were taken to the MRI waiting area. Here they were instructed to place all jewellery, coins, and other paramagnetic or loose items into a locker, after which they were settled into the scanner by the duty radiographers. During this setup, radiographers fitted participants with the following: (i) ear protection to minimise scanner noise; (ii) a pair of headphones through which to listen to the soundtrack of the animated DVD that would be played to them during their scan; and (iii) a pulse oximeter, clipped onto two fingers of the left hand, to monitor the pulse during the cardiac gated DT-MRI sequence. Once the participant was comfortable in the scanner, the radiographers returned to the imaging suite to initiate the scanning sequences. Throughout the session participants were able to communicate with both me and the duty radiographer via a microphone and speaker system within the scanner. The whole session (including sequences not reported in this thesis) lasted for between forty to fifty-five minutes, depending on whether any scans required repeating due to participant head movement.

Following the MRI session participants collected their belongings from their locker, were offered refreshments and a short break, and then taken into an interview room. During this session IQ testing (WASI) was completed, plus – where indicated (see Group allocation and Recruitment Flowchart below) - the K-SADS-PL and PCL-YV interviews were administered. Finally, on completion of testing, participants were thanked for their time and reimbursed with £50 in cash and completed a claim form for any receipted travel expenses.

4.9 Group allocation

Prior to testing participants, I had scored parent and self-report postal questionnaires. It was from these scores that grouping of participants was first initiated, in accordance with methods used in previous neuroimaging studies of antisocial behaviour in young people (Finger, Marsh et al. 2008; Jones, Laurens et al. 2009; see Figure 4.3); this was then completed as follows.

After ensuring general exclusion criteria were not met (see above), all 43 remaining males were screened for conduct problems using the parent- and self-report version of the SDQ. Where boys fell within the 'abnormal' range on the conduct problems scale on either the self- or parent report, they were administered the CD and ODD sections of the K-SADS-PL interview from which they could be given a research diagnosis of Conduct disorder. Where this threshold was not met, participants formed the healthy control group (n=14). Participants for whom informant-report by a parent/guardian was not available (n=2), were given the K-SADS-PL interview irrespective of their self-report SDQ conduct problems score. This interview was not administered to parents of participants, due to difficulties with their availability to be interviewed. However, having previously questioned them on the screening version to the K-SADS-PL interview during the recruitment phase, it was possible to ascertain a good understanding the type and extent of boy's behaviour problems in order for cross-referencing to be possible where there were concerns regarding the

validity of boys' responses. No boys met criteria for ODD in the absence of CD; all participants were unmedicated.

In addition to grouping participants into major categories of CD or healthy control, the CD group were subdivided into those with high levels of psychopathic traits and those with low levels. For this, a composite score of 20 or above on parent- and self-report versions of the APSD indicated the possible presence of these traits; this method has previously been used in a similar sample (Dadds, Perry et al. 2006). Those scoring below 20 were allocated to the CD group (n=12), while for those scoring greater than 20 the PCL-YV interview was administered. Individuals with a score above 20 on that measure were assigned to the 'CD with Psychopathic traits' group (n=13). Two individuals scoring below 20 (n=2), indicative of low levels of psychopathic traits, were allocated to the CD (without psychopathic traits) group.

The study's two major groups (CD and healthy controls) did not differ significantly in age, ethnicity, full scale IQ, education, household income, and drug use history. Furthermore, as mentioned above, both groups contained an equal number of individuals who had ever received a diagnosis of ADHD (n=2). Also they did not significantly differ in level of ADHD symptoms as measured by the SDQ 'hyperactivity/inattentiveness' subscale. No participants reported any other psychiatric history (excluding CD, or referrals for anger management); and all boys spoke English as their first language and were right handed as assessed by the Edinburgh Handedness Inventory (see Measures and Appendix 14; Oldfield 1971).

Table 4.3: Group characteristics

	Conduct disorder (n = 27) Mean (SD)	Healthy controls (n=16) Mean (SD)	P value (t-test)
Age in years	16 (2)	16 (2)	0.858
Mean FSIQ	99 (8)	103 (10)	0.098
Conduct problems (SDQ)	6 (2)	3 (1)	0.000**
Hyperactivity (SDQ)	7 (2)	6 (2)	0.375
Emotional Problems (SDQ)	4 (3)	3 (2)	0.061
Peer Problems (SDQ)	4 (2)	3 (2)	0.034*
Prosocial Behaviour (SDQ)	7 (2)	8 (1)	0.161
Total problems (SDQ)	18 (5)	12 (5)	0.000**
Callous-unemotional traits (APSD)	7 (2)	5 (2)	0.012*
Narcissism (APSD)	8 (3)	6 (3)	0.017*
Impulsivity (APSD)	7 (2)	6 (2)	0.048*
Total score (APSD)	25 (7)	19 (6)	0.005**
Ethnicity (%)	n=27	n=16	Chi^2
White	52	63	0.717
Black/African-Caribbean	33	25	^0.735
Other	15	13	^1.000
Annual income (%)	n=17	n=10	Chi^2

Below £18,000	47	10	^0.091
£18,000-£25,000	24	40	^0.415
£25,000-£43,000	24	30	^1.000
Above £43,000	6	20	^0.535
Substance use (%)	n=20	n=15	Chi^2
Cannabis - ever used	60	40	0.407
Cannabis – used in past month	(n=12) 50	(n=6) 67	^0.638
Alcohol – ever used	75	100	^0.057
Alcohol – used in past month	(n=15) 73	(n=15) 67	^1.000
Cocaine – ever used	7	0	^0.496
Amphetamine – ever used	7	0	^0.496
#Any other drug – ever used	15	7	^0.619

*SDQ – Strengths and Difficulties Questionnaire; APSD – Antisocial Process Screening Device; SD – Standard Deviation; *p<0.05; **p<0.01; #Excluding alcohol and cannabis; ^Fishers exact test*

Figure 4.3: Recruitment flow-chart

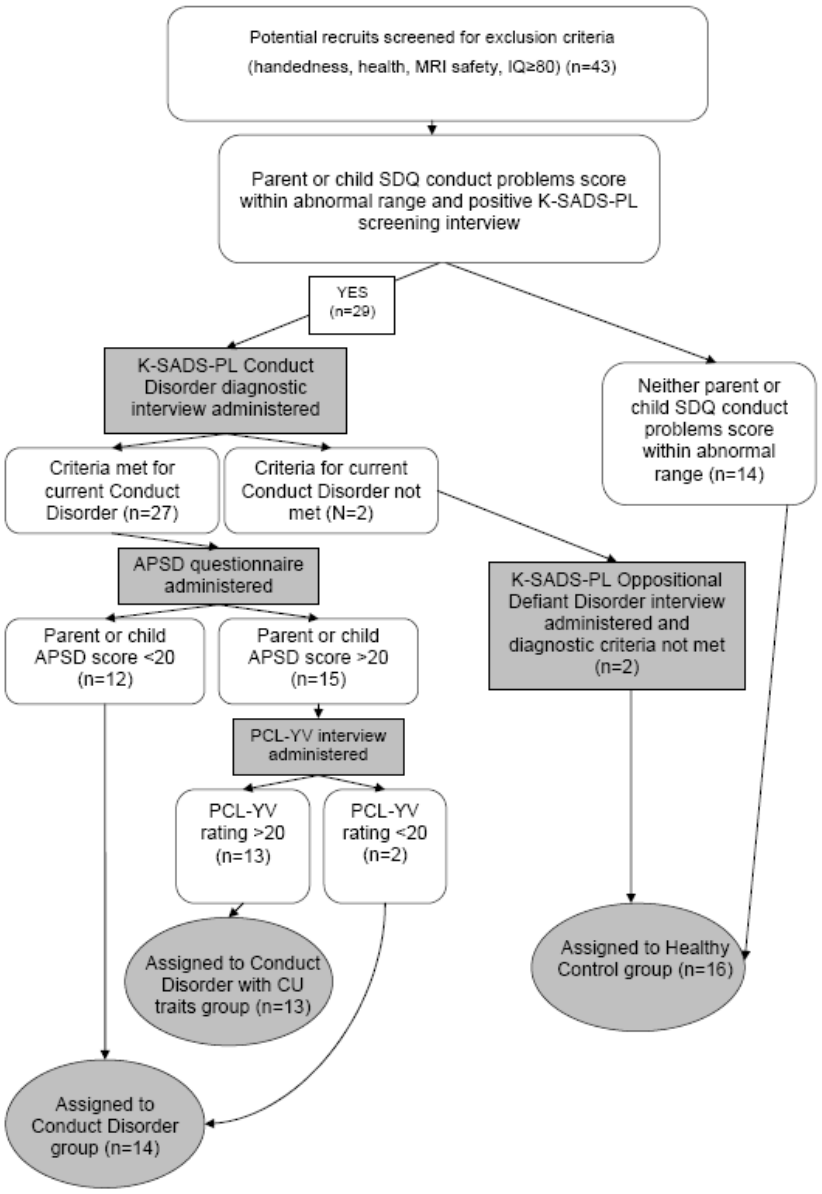


Diagram to show how participants were allocated to the three study groups: (1) the CD group - scored within the abnormal range of either the informant or self-report conduct problems subscale of the Strengths and Difficulties Questionnaire (SDQ) and satisfied criteria to meet a research diagnosis of conduct disorder on the K-SADS-PL interview. Individuals and informants completed the Antisocial Process Screening Device (APSD), and those scoring above 20 on either version were given the Psychopathy Checklist Youth Version (PCL-YV) interview. Those scoring below 20 joined the CD group; (2) individuals who met diagnostic criteria for CD and scored above 20 on the PCL-YV were allocated to the CD with Callous-unemotional traits group; and (3) individuals allocated to the healthy control group did not meet a research diagnosis of ODD or CD on the K-SADS interview.

Table 4.4: Proportion of individuals within Conduct disorder group meeting each K-SADS-PL Conduct disorder item

K-SADS-PL Conduct disorder items	% boys in CD group (n=27)	
	met criteria	met at sub-threshold level
Lying	54	39
Truanting	54	11
Physical fighting	89	7
Bullying, threats or intimidation	75	14
Stealing	57	18
Vandalism	39	18
Breaking and entering	29	11
Aggressive stealing	29	4
Fire setting	7	14
Staying out overnight	18	14
Running away from home	21	4
Weapon use	61	11
Physical cruelty	61	11
Forced sexual activity	0	4
Animal cruelty	11	7

4.10 Neuroimaging

4.10.1 Diffusion Tensor Magnetic Resonance Imaging (DT-MRI)

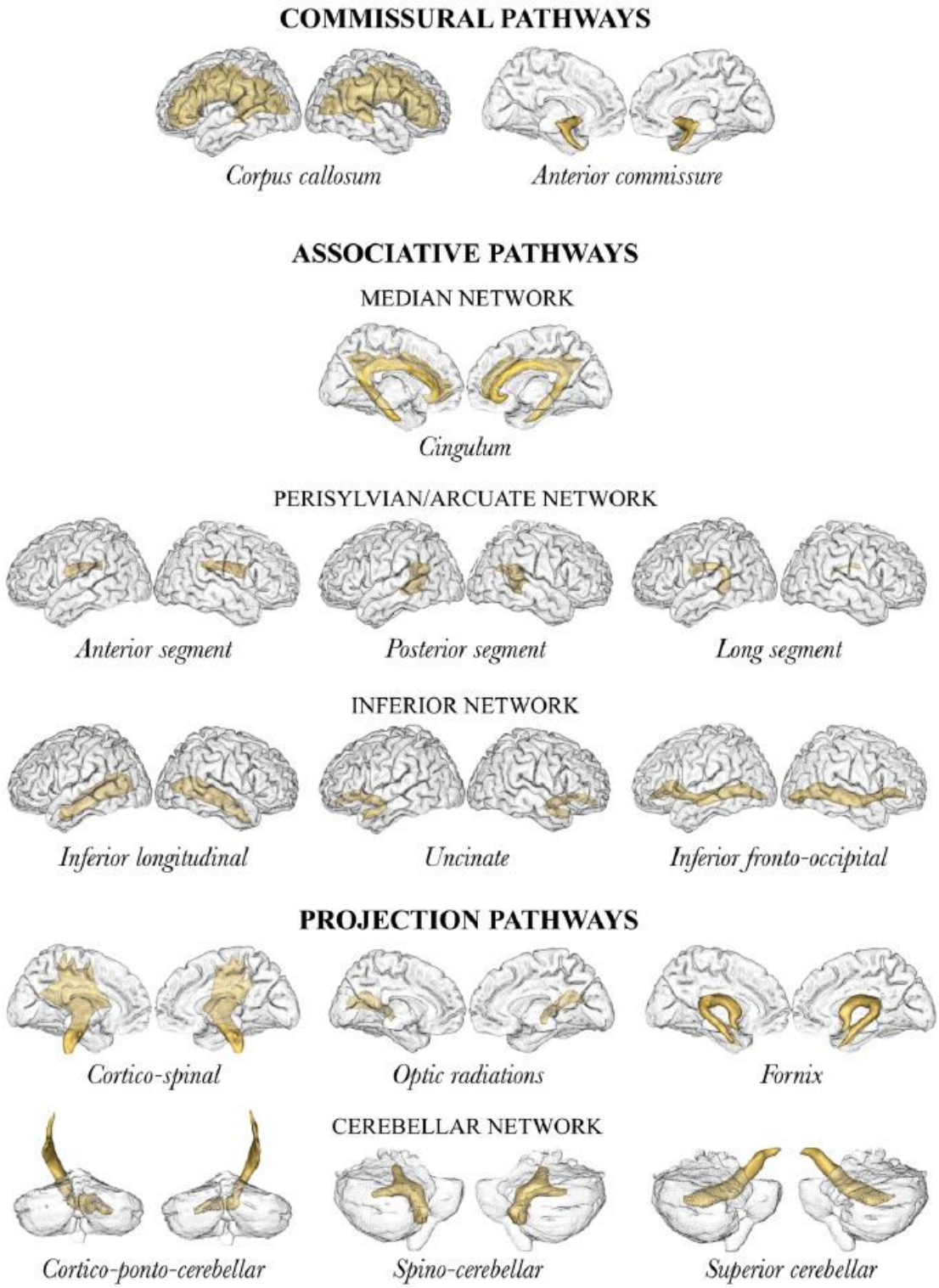
DT-MRI is a relatively recent neuroimaging technique that harnesses the diffusion properties of water molecules within tissue to create three dimensional reconstructions of the white matter tracts that comprise the inter-cerebral connections. I will outline the methods used to analyse these data after first describing the basis of DT-MRI to investigate the microstructure of white matter tracts.

4.10.1.1 White matter tracts

White matter tracts are bundles that are composed of neuronal processes. Their white colour comes from the myelin sheath that covers individual axons within the tracts. Myelin is a lipid based layer that begins to develop at around the 28th week of gestation, and continues until early adulthood (Marsh, Gerber et al. 2008). Myelin is essential for efficient neurotransmission to take place, and demyelinating disorders (e.g. multiple sclerosis) cause significant functional impairments. White matter tracts, also known as fasciculi, are categorised into three types, based on their directionality: commissural, projection, and association (see Figure 4.4). Commissural tracts connect the left and right hemisphere, and include the anterior and posterior commissures. The largest commissural tract is the corpus callosum, and patients in whom this tract has

been severed are unable to combine information between the hemispheres; this is called the ‘split brain’ phenomenon (Gazzaniga 2005). Projection tracts connect the midbrain and spinal cord with, first, the cerebral cortex, and also the cerebellum. For example, the corticopontine tract projects from the cerebellum to the pons and the cortex. Finally, association tracts provide connections between regions within the same hemisphere. Association fibres are subcategorised as ‘long’ or ‘short’ fibres, depending on whether they connect distant or neighbouring areas, respectively. The longest of these is the superior longitudinal fasciculus, which joins frontal regions with the temporal, parietal and occipital lobes. Further association tracts include the uncinate fasciculus, inferior fronto-occipital fasciculus, and the inferior longitudinal fasciculus; these will be described further below.

Figure 4.4: White matter tracts



Catani & Thiebaut de Schotten, 2008

4.10.1.2 The basis of DT-MRI

White matter tracts can be visualised through DT-MRI, which reconstructs images through gauging the diffusion of water molecules, which takes place constantly within all living tissues. Brain tissue, composed of mostly water, contains several different structural components – e.g., white matter and grey matter. Cellular properties differ between tissue types, and these properties determine the relative ease with which diffusion can take place. For example, white matter tracts contain axons that are arranged in an ordered, lengthwise manner, while cells within grey matter are arranged more randomly (Jones 2008). Consequently diffusion differs in its directionality; unidirectional diffusion is known as 'anisotropy', whereas diffusion that occurs in any direction (i.e. free from restrictions, such as in grey matter or cerebrospinal fluid) is 'isotropic'. In these terms, white matter tracts generate anisotropy by hindering diffusion across the tract while not affecting diffusion in the dominant, longitudinal direction. Anisotropy is measured on a scale of 0 to 1, which indicate fully isotropic to fully anisotropic, respectively.

Based on the measures of diffusion and anisotropy, DT-MRI of white matter enables micro- and macro-structural cellular organisation of tracts to be inferred through observing the way diffusion is occurring. Properties such as membrane permeability and the density with which fibres are packed together contribute to differences in diffusion properties between tracts. For example, neural damage in patients with multiple sclerosis can be inferred from DT-MRI scans, where

increases in values that reflect membrane permeability (e.g. perpendicular diffusivity, see below) indicate a loss of myelin (Harrison, Caffo et al. 2011).

The diffusion properties of white matter are ascertained through the acquisition of diffusion weighted images using MRI. MRI techniques enable images of the internal structures of living subjects to be acquired non-invasively. During basic MRI the scanner's magnetic field aligns the protons (1H) within the water molecules of brain tissue. The application of radio frequency (RF) pulses excites these protons, which subsequently relax back to their previous state. The relaxation of protons results in the emission of RF signals, which are received and converted into images. The difference in relaxation rate of protons in different areas of the brain reflects tissue properties, and these cause differences in signal magnitude that in turn create contrast in the MR images. Diffusion weighted images are created through the manipulation of several parameters (e.g. the number of RF pulses applied and the time between RF applications) and the use of different magnetic field gradients. Thus, depending on their spatial location, excited protons are exposed to different magnetic strengths. This results in different frequencies of proton spin that ultimately enable the labelling of water molecules at an initial time point, and the tracking of their diffusion rates to be calculated at a subsequent time point. In this way, water diffusivity inside the brain is ascertained by collecting diffusion measurements from many (usually six) directions (see Jones 2008 for fuller description).

The direction in which the maximum rate of diffusion occurs within tissues is estimated by application of the diffusion tensor, which is a mathematical model comprising diffusion values derived from each of the directions from which the scan has acquired diffusion weighted images, which in this study was 6 (Figure 4.5).

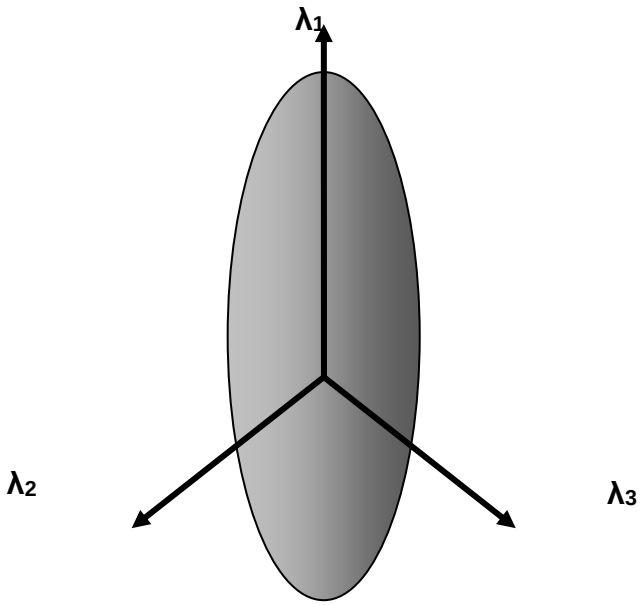
Figure 4.5: The Diffusion Tensor

$$\mathbf{D} = \begin{bmatrix} D_{xx} & D_{xy} & D_{xz} \\ D_{xy} & D_{yy} & D_{yz} \\ D_{xz} & D_{yz} & D_{zz} \end{bmatrix}$$

When applied, the diffusion tensor fits the diffusion-weighted data into an ellipsoid shape (Figure 4.6). Due to the symmetry of the tensor we obtain two sets of values: eigenvectors, which are the three major axes of the ellipsoid (v1, v2, v3), and eigenvalues, the diffusivity occurring along the directions of the three eigenvectors (,λ1, λ2, λ3). The major eigenvalue (λ1) quantifies maximum diffusivity (i.e. axial, or parallel, diffusivity), with the smaller two representing radial, or perpendicular, diffusivity. Therefore, perfect isotropy would result in an ellipsoid that more closely resembles a sphere (i.e. with equal rates of diffusion occurring along all three eigenvectors), while perfect anisotropy would result in a straight, one-dimensional line (i.e. with strongly unidirectional

diffusivity). Finally, the values of the eigenvectors are used to calculate the different anisotropy indices that enable white matter quantification, such as fractional anisotropy (FA) and perpendicular diffusivity (D_{perp}). These indices and their proposed functional significance are described here.

Figure 4.6: The Diffusion Tensor Ellipsoid



Representation of the elliptical shape of diffusion rates estimated through application of the diffusion tensor. The ellipsoid embodies the shape of the extent to which protons with spin are equally likely to diffuse. Point v_1 represents the dominant orientation of diffusion within the medium.

4.10.2 DT-MRI values

A number of anisotropy indices are obtained using information from the diffusion tensor ellipsoid. These indices are understood to reflect different aspects of white matter micro- and macro-structure that contribute to the integrity and organisation of white matter tracts; these values form the basis of statistical analyses conducted in DT-MRI studies. These four values are: fractional anisotropy, mean diffusivity, parallel (axial) diffusivity, and perpendicular (radial) diffusivity.

4.10.2.1 Fractional anisotropy (FA)

FA quantifies water molecule diffusion that is occurring longitudinally along white matter tracts, parallel to the axonal bundle; the FA value ranges between 0 and 1, indicative of perfectly isotropic (random) versus perfectly anisotropic (unidirectional) water molecule diffusion, respectively (Pierpaoli and Basser 1996). FA is derived from an estimation of the difference in diffusivity across the three eigenvalues. For example, where there is no difference between the three values FA would be zero; this would correspond to total isotropy. Where there is a difference in only one direction (i.e. v1) FA would be totally anisotropic and have a value of one. This value provides a proxy measure of tissue integrity (Horsfield and Jones 2002; Mori and Zhang 2006). Although there is currently no consensus as to what the underlying microstructural basis for FA value is, it is believed that a combination of myelin properties and inter- and

intra-axonal factors, such as axon diameter and fibre number, influences anisotropy value (Beaulieu 2009; Paus 2010). Further, FA increases with age during childhood and adolescence (Lebel, Walker et al. 2008).

4.10.2.2 Mean diffusivity (MD)

Mean diffusivity is the average of the 3 eigenvalues and this provides a description of the mean mobility of water in each voxel. The diffusion of water molecules is affected by tissue type and intercellular components that may impede diffusion, such as disorganised microtubules within white matter tracts.

4.10.2.3 Parallel (axial) diffusivity (D_{parr})

D_{parr} is the absolute value of diffusivity along the principle eigenvector. However, the eigenvalue derived from the tensor model makes the assumption that there is only one fibre, thus care needs to be taken in the interpretation of this value as we know that there are crossing fibres, those that pass through the main tract at an angle, and that these affect the D_{parr} value.

4.10.2.4 Perpendicular (radial) diffusivity (D_{perp})

D_{perp} reflects the diffusion of water molecules that is occurring radially, across – rather than along - the fibre bundle. Thus, it indexes the level of membrane integrity along the length of the fibre. Increased D_{perp} occurs with demyelination, and is thus considered a marker for reduced membrane integrity

that has its basis in reduced myelin content as well as intra-axonal factors (Beaulieu 2009). Further, reduced D_{perp} is seen in typical brain maturation and is associated with increasing FA (Lebel, Walker et al. 2008), which suggests its association with myelination. Finally, D_{perp} is also found reduced in proportion to axon numbers, where there is increased extracellular space (Beaulieu 2009).

4.10.2.4.1 DT-MRI acquisition

Each DT-MRI image was acquired using a GE Signa HDx 3.0T MR scanner (General Electric, Waukshua, WI, USA), with actively shielded magnetic field gradients (maximum amplitude 40 mT m-1). The body coil was used for RF transmission, and an 8 channel head coil for signal reception, allowing a parallel imaging (ASSET) speed up factor of two. Each volume was acquired using a multi-slice peripherally-gated doubly refocused spin echo EPI sequence, which had been optimised for precise measurement of the diffusion tensor in brain parenchyma, from 60 contiguous near-axial slice locations with a voxel size of 1.85 x 1.85 x 2.4 mm. The TE was 104.5 ms while the effective TR varied between subjects in the range 12 and 20 RR intervals. Based on the recommendations of Jones et al (Jones, Williams et al. 2002), the maximum diffusion weighting was 1300 s mm-2, and at each slice location 4 images were acquired with no diffusion gradients applied, together with 32 diffusion-weighted images in which gradient directions were uniformly distributed in space. The sequence ran for approximately 15 minutes, although an additional thirty minutes of scanning acquired data contributing towards a larger study.

4.11 Tractography analysis

4.12 DT-MRI tractography

Tractography is a method of investigating white matter integrity in specific predetermined tracts of interest, in hypothesis-driven studies. This contrasts with whole-brain investigations, which do not use *a priori* tracts of interest, but instead examines all the white matter pathways within the entire brain to find areas of significant difference between groups. This section outlines the steps I followed to conduct the DT-MRI tractography analysis in Chapter 5 (Study 1 - CD) of the thesis. Tractography was also used in Study 2 (Chapter 7), and the same preprocessing and dissection steps that are given below were followed in that study.

4.12.1 Preprocessing

After collecting the scanning data using the DT-MRI acquisition parameters outlined above, a number of preprocessing steps needed to be followed. Firstly, I converted scans from UNC format in which they were acquired, into ANALYZE format, in four dimensions. Next, I converted the images into NIfTI format, and quality checked them using the FSLview lightbox function, part of the FSL software package (www.fmrib.ox.ac.uk/fsl). Data were next imported into the ExploreDTI program (Leemans, Jeurissen et al. 2009) and checked to

ensure fibre orientations within the FA maps produced were correctly oriented according to x, y and z directions, as in the gradient tables. In order to do this, I used the 'drawing tool' to select the corpus callosum on the sagittal plane, from which the program calculates the tracts. Completion of this stage resulted in data being converted from NIfTI format into MAT format. All files were then selected for motion distortion and Eddy current correction.

After correcting the data, the diffusion tensor was estimated following removal of outlier data (RESTORE function). Next white matter tracts in each participant's data-set were mathematically reconstructed by tracking along the major eigenvector within each voxel and joining these together within the whole brain. Parameters used to select seed voxels were: fractional anisotropy (FA) ≥0.2; Euler integration used to propagate streamlines (Basser, Pjevic et al. 2000); tractography algorithm step size set to 0.5mm; and, finally, for tractography to stop where FA<0.2 or where the angle between consecutive steps exceeded 30 degrees. Finally, the following Diffusion Tensor maps were estimated and these tractography data were exported into TrackVis v0.4.3 software.

Table 4.5: Outputs generated during DTI preprocessing

FA	fractional anisotropy
MD	mean diffusivity
FA-colour	colour fractional anisotropy map
D_{perp}	perpendicular diffusivity
D_{parr}	parallel diffusivity
MeanDWI	mean diffusion weighted image

The Diffusion Tensor maps are whole brain estimates, therefore individual tract values can only be ascertained through further analysis. TrackVis software (Wang and Wedeen 2006-2010) facilitates the visualisation and in vivo dissection of white matter tracts using a deterministic fibre tracking approach. As such, tracts are dissected by placing seed points, or regions of interest (ROIs), within the virtual 3-dimensional space corresponding to points intercepted by the particular tract of interest. The estimated FA map is first opened, and this is used as an anatomical guide by which to place ROIs. A ROI can be either inclusive, whereby all fibres passing through that point are incorporated into the tract, or exclusive, whereby all fibres intercepting the ROI are omitted. I received full training in tractography using TrackVis software following attendance of the Natbrainlab tractography workshop, Institute of Psychiatry. I achieved significant inter- and intra-rater reliability in dissection of the three tracts examined – and was blind to clinical groupings. The steps I followed to dissect each of the three tracts that were examined in both studies

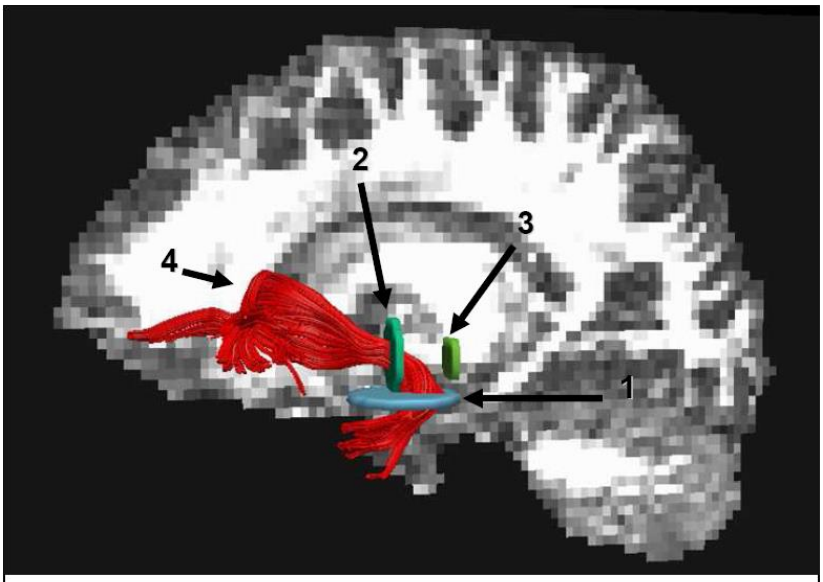
within the thesis (Chapter 5 – CD; and Chapter 7 – prenatal stress) will now follow.

4.12.2 White matter tracts

4.12.2.1 Uncinate fasciculus

The first of the three tracts dissected, and the tract of interest, was the uncinate fasciculus (UF). This is a limbic association tract that connects the amygdala and anterior temporal lobe with the medial and lateral aspects of the orbitofrontal cortex. Association fibres join cortical regions within the same hemisphere and vary in length. The UF is a short, C-shaped tract that was dissected through the placement of two regions of interest (ROIs). The first ROI was placed on the axial slice at the level of the medial temporal lobe, and the second was placed coronally slightly posterior to the external capsule. Short fibres that did not enter either termination of the tract were excluded, as were long fibres extending to regions outside the frontal and temporal lobes. These fibres were omitted through placement of exclusion ROIs.

Figure 4.7: Dissection steps for uncinate fasciculus tract

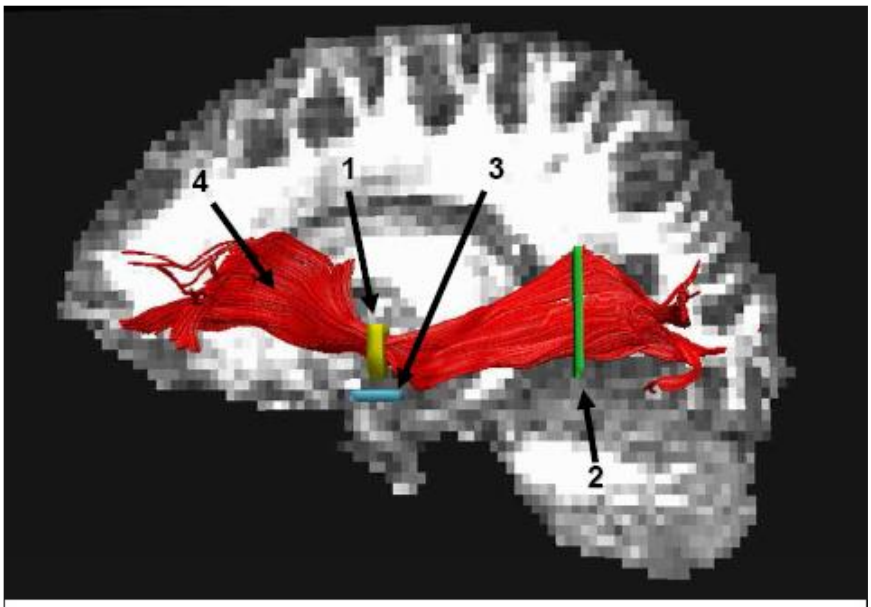


ROI - region of interest; 1 - first, axially placed ROI (inclusive); 2 - second, coronally placed ROI (inclusive); 3 - third ROI (exclusive); 4 - dissected uncinate fasciculus tract

4.12.2.2 Inferior fronto-occipital fasciculus

The Inferior fronto-occipital fasciculus (IFOF) was the first of two control tracts examined in both tractography studies; it was selected because it too is an association tract that extends from the frontal lobe, and originates within the same region as the uncinate fasciculus. However, unlike that tract, the IFOF terminates in the occipital lobe, thus bypassing the limbic region; making this a good choice of control tract as it is not involved in limbic connection. Dissection of the IFOF used two ROIs, the first placed at the same point as the coronal ROI for the uncinate fasciculus (see above), and the second placed within the occipital lobe. As before, fibres were excluded that did not extend between both terminations by placing additional ROIs.

Figure 4.8: Dissection of inferior fronto-occipital fasciculus

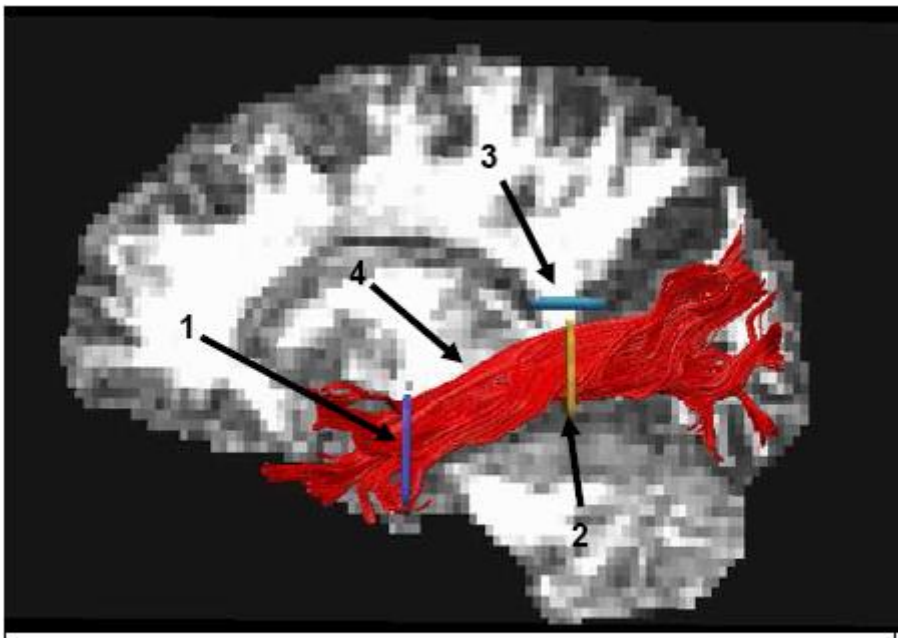


ROI - region of interest; 1 - first, coronally placed ROI (inclusive); 2 - second, coronally placed ROI (inclusive); 3 - third ROI (exclusive); 4 - dissected inferior fronto-occipital fasciculus tract

4.12.2.3 Inferior longitudinal fasciculus

The inferior longitudinal fasciculus (ILF) was the second control tract that I delineated. This tract, in common with the IFOF, shares one originating point (within the anterior temporal lobe) with the uncinate fasciculus; however, the ILF is more posterior, towards the occipital lobe. The two ROIs used to dissect this tract were those already used in dissection of the previously dissected two tracts. These were the coronal ROI of the uncinate fasciculus, and the posterior ROI of the IFOF. Again exclusion ROIs were placed to remove superfluous fibres.

Figure 4.9: Dissection of inferior longitudinal fasciculus



ROI - region of interest; 1 - first, coronally placed ROI (inclusive); 2 - second, coronally placed ROI (inclusive); 3 - third placed ROI (exclusive); 4 - dissected inferior longitudinal fasciculus tract

4.12.3 Statistics

In order to assess between group differences in the values of the three dissected white matter tracts, I used Statistical Package for the Social Sciences (SPSS) software to first apply a repeated measures analysis. This used the within subjects variables 'tract' (UF, IFOF, ILF) and 'hemisphere' (left, right); and the grouping variable of interest 'group' (e.g. 'Conduct disorder' or 'Control group') as the between subjects variable. This analysis tested for significant between group differences in FA and D_{perp} in the uncinate fasciculus and the two control tracts. *Post hoc* analyses were carried out to identify significantly

differing FA/D_{perp} values between boys with CD and healthy controls using one-way analysis of variance (ANOVA). Due to multiple tests performed at this stage, the results required correction for multiple comparisons, and so a Bonferroni test was carried out. This requires that the significance value at which the null hypothesis would normally be rejected (e.g. $p<0.05$), be divided by the number of comparisons made. In this case, as six comparisons were carried out the necessary value below which between groups were required to fall was 0.008 (i.e. 0.05÷6).

Further *post hoc* testing was carried out where significant FA or D_{perp} differences were detected, in order to determine whether significant differences in these indices were associated with clinical and behavioural measures. Here, one-tailed correlations were carried out testing the hypothesis that worse behaviour correlated with greater differences. Also, in order to examine the effect of age on any white matter measures that significantly differed between groups I applied a two-tailed Pearson's correlation. This was carried out within each group separately to give coefficients. Last, to determine whether there were significant differences in the effect of age I compared these two coefficients using, Z-observation (Z_{obs}) analysis. This requires that each r-value is first converted into a z-score, and then a Z_{obs} value is calculated from the following equation:

$$Z_{obs} = \frac{Z_1 - Z_2}{\sqrt{\frac{1}{N_1-3} + \frac{1}{N_2-3}}}$$

Where a Z_{obs} falls between -1.96 and 1.96 this indicates no significant difference between coefficients, whereas values outside this range denote a significant difference.

4.13 TBSS analysis

4.14 Tract based-spatial statistics analysis

The second type of white matter analysis used in this thesis (Study 1 - Chapter 6) was tract-based spatial statistics (TBSS). Whereas tractography dissects individual tracts of interest on the basis of *a priori* hypotheses, TBSS examines global patterns of white matter integrity in a groupwise, voxel-wise manner. The advantage of this is that it does not restrict the investigation of FA differences to only white matter tracts with established relevance to the population; therefore, TBSS can potentially highlight white matter differences in regions not previously known to be of importance in the particular study cohort (i.e. in non hypothesis

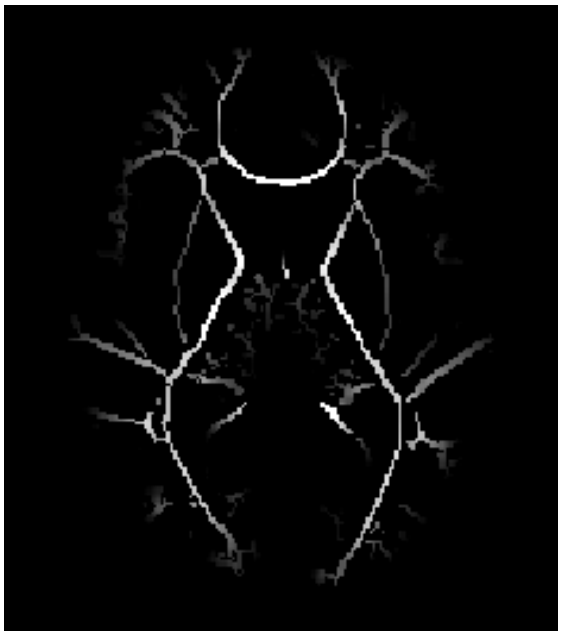
driven investigations). Furthermore, also in contrast to DTI tractography, this method is fully automated so requires no operator input such as the lengthy process of delineating, by hand, predefined regions of interest. However, as with other whole-brain image analysis techniques TBSS is a more conservative method, meaning that where a region of interest approach only compares a small number of tracts (e.g. one tract of interest versus one control tract), TBSS sequentially compares every voxel to its corresponding voxel within each participant's brain. Thus, differences seen using TBSS can be viewed as more robust than tractography, as differences need to withstand multiple statistical comparisons.

In order to perform TBSS analysis, a white matter 'skeleton' is produced upon which each data set is compared, in order to find areas of significant difference. The skeleton represents the white matter core of each tract upon which all subjects' brains overlap (see Figure 4.10). The steps by which the skeleton is produced and analysed is achieved using FSL (Fmrib Software Library, www.fmrib.ox.ac.uk/fsl), and these are outlined below. Specific preprocessing was not required for this method as the FA maps and MD maps generated from the tractography preprocessing step (described previously) were used for the following processing steps.

4.14.1 TBSS processing

The first step of TBSS processing requires that each participant's FA map is transformed into standard stereotactic space, which can either be a standard template or - as was the case in the current study - a study specific template. This was the FA map most representative of all FA images within the sample. I chose the option of creating my own template because the standard templates available (e.g. MNI 152) are based on adult FA maps and are as such not appropriate for use with a developmental sample. Next, all the FA maps are collapsed into one image and then averaged to give a mean FA map for the whole sample; from this the average core 'skeleton' is created. By using only the core of the sample's white matter the peripheral tract regions are not involved in further analysis, thus removing partial volume effects.

Figure 4.10: Example of an averaged FA skeleton



After the mean skeleton is produced, skeleton images of each participant's data (FA map) are produced, and are then projected onto the mean skeleton to identify voxels where FA value differs significantly between these skeletons using voxel-wise statistics. In order for these statistical comparisons to be made, a design matrix is required that specifies subject grouping details and covariates. I created a design matrix that contained the following variables: CD, control, age by group, IQ by group. I will outline the design and its constituent contrasts here.

4.14.2 Statistical analysis

The design matrix contained the following columns:

Table 4.6: Values specified within design matrix

Design matrix value	Coding
CD	1 (0 for controls)
Control	1 (0 for CD)
Age CD	demeaned age of each CD participant
Age control	demeaned age of each control participant
IQ CD	demeaned full scale IQ (FSIQ) score for each CD participant
IQ control	demeaned FSIQ score for each CD participant

IQ – Intelligence Quotient; FSIQ – full scale IQ; CD – Conduct disorder

Age and IQ were demeaned (mean value subtracted from each value). Within the design it was necessary to specify which contrasts the statistics tool (randomise) will perform; I specified ten contrasts, to calculate the following statistical differences in FA:

Table 4.7: Contrasts examined within TBSS analysis

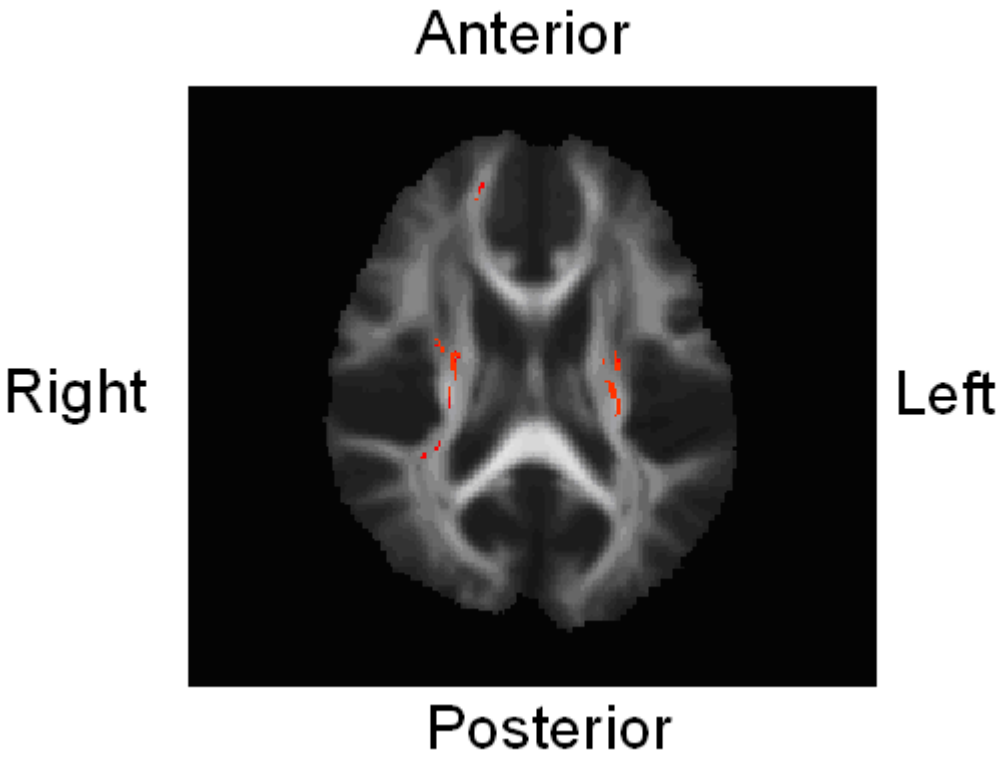
	Contrast	Description
1	CD > controls	areas with greater FA in CD group
2	CD < controls	areas with lower FA in CD group
3	Age CD +	areas where FA of CD group positively correlated with age
4	Age CD -	areas where FA of CD group negatively correlated with age
5	Age controls +	areas where FA of controls positively correlated with age
6	Age controls -	areas where FA of controls negatively correlated with age
7	IQ CD +	areas where FA of CD group positively correlated with IQ
8	IQ CD -	areas where FA of CD group negatively correlated with IQ
9	IQ controls +	areas where FA of controls positively correlated with IQ
10	IQ controls -	areas where FA of controls negatively correlated with IQ

IQ – Intelligence Quotient; CD – Conduct disorder

When specifying calculation of these contrast, I selected the statistical command that, within the same step, corrected for multiple comparisons using threshold free cluster enhancement with a specified significance level of $p<0.05$. This final step resulted in the generation of ten images – each of which showed coloured regions representing areas of significant difference in FA corresponding to the particular contrast examined.

After producing these contrast images, I used the fslview function of FSL software to visualise the data. First I opened the mean FA map of the group and set the minimum and maximum thresholds to 0 and 0.6, respectively, in order to visualise these easily. I then opened the contrast image over the FA map, setting the minimum and maximum thresholds to 0.95 and 1, respectively. These values ensured that only areas of difference that were significant at p=0.05 (i.e. 0.95 threshold) were shown within the contrast. I set the colour that these significant regions would be displayed in to red, to ease viewing.

Figure 4.11: Example of TBSS contrast image

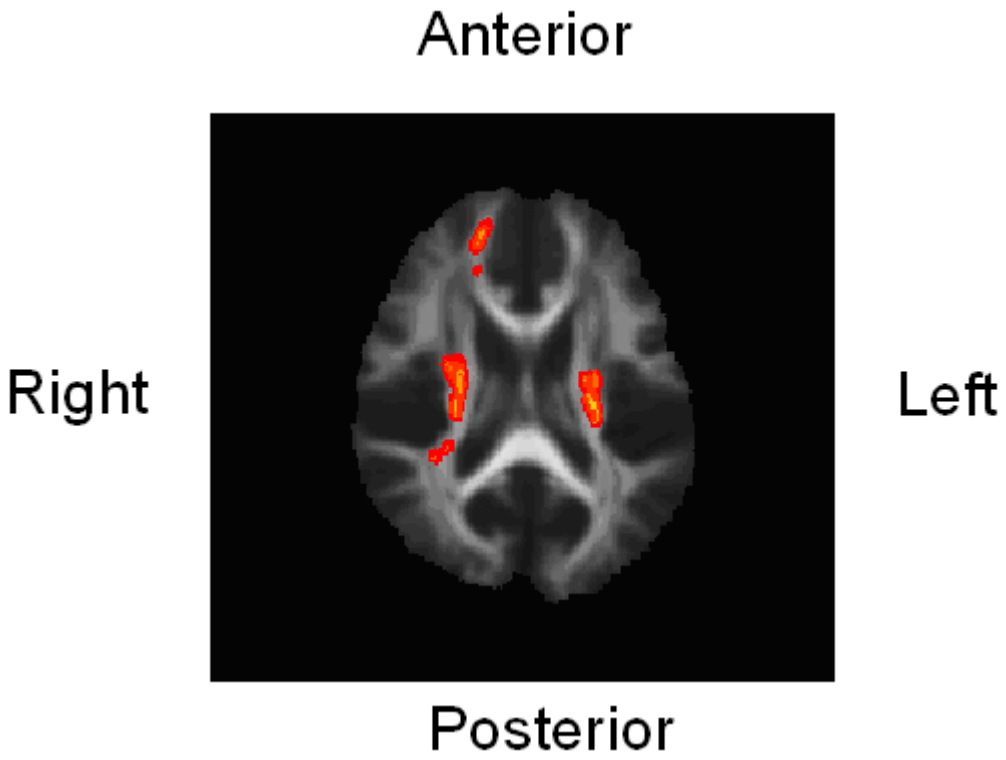


Finally, in order to facilitate the viewing of these regions further, I used the FSL 'fill' command to 'thicken' each of the regions. This command:

Tbss_fill [name of contrast file] 0.95 mean_FA tbss_fill

resulted in this thickened version of the above image:

Figure 4.12: Example of 'thickened' TBSS contrast image



I report the results of TBSS analysis with reference to a white matter atlas (Catani and Thiebaut de Schotten 2008) and the John Hopkins University (JHU) white matter tractography atlas that is available within the FSL package (Hua, Zhang et al. 2008). While current white matter atlases are based on adult data, they can be used to identify tracts in developmental populations (Jou, Mateljevic et al. 2011), although limitations should be noted. The areas of difference were thus reported both visually and descriptively within the TBSS results section (Chapter 6). This served as an exploratory investigation of white matter differences between CD and control groups.

4.15 Study 2

As outlined in the overview at the start of this chapter, the participants who were investigated in Study 2 belonged to a pre-existing cohort that were recruited between 2001 and 2004 by researchers at Imperial College London, under the Principal Investigator (PI) Professor Vivette Glover. I was given access to this cohort in order to undertake a neuroimaging study in collaboration with the PI. The data included within this thesis constitute the first part of this larger study, which remains ongoing. This study utilised three types of data: (1) maternal prenatal measures collected at the initial stage of recruitment; (2) behavioural data collected at the second stage when infants were approximately 17 months old; and (3) neuroimaging and behavioural data collected at approximately age 8 as part of this thesis. Therefore, the description of methods within the study will focus mainly on those data that I have collected, and which forms the focus of the study reported in Chapter 7.

4.16 Ethical issues and Ethical approval

As a first step in undertaking this study, I complied an ethics application and attended the Brent Research Ethics Committee meeting in July 2009 (see Appendix 15 - Ethics number: H/0717/09), where the application was approved. The main ethical consideration raised by the Committee was the possibility that children may experience anxiety during scanning. It was deemed important for

special attention to be paid to preparing participants for their scanning session, and for this reason I wrote a short script and storyboard (see Appendix 16) to be used to create a short video presentation. I contacted staff at the Institute of Psychiatry Audiovisual department, who kindly recorded the video for me, and I organised for a mother and child to pose as participants and be filmed. I sent a URL link to the video to parents for them to view with their child prior to their testing session (http://www.admin.iop.kcl.ac.uk/MRIvideo.mpg) in order to familiarise them both with the testing schedule and with the MRI scanning procedure. In addition, provision was made whereby a parent would be able to remain with their child in the room during scanning, so long as they too were MR compatible.

A further recommendation made by the Ethics Committee was that the General Practitioner of each child be informed of his/her participation in the study. It was deemed necessary that a letter be sent to the GP in order to remain on the child's notes should the child experience any adverse effects as a result of having undergone MRI (e.g. nightmares, anxiety, etc; see Appendix 17).

One final question raised by the committee was the method by which the study would disseminate research findings to participants. It was decided that to minimise distress should findings suggest antenatal stress contributes negatively to neurodevelopment, data should be reported generally and counselling offered to mothers by a dual trained obstetrician and psychiatrist (Dr. Michael Craig).

4.17 MRI scanner training

A further measure that I took in order to ensure that children were comfortable within the scanner, was to devise a forty-five minute mock scanner training session that used a schedule of positively rewarded exercises to: a) prepare children for the scanning environment; b) allow them to habituate to remaining still for the scan duration; c) train them on the fMRI tasks they would be later performing (these tasks are not reported in this thesis); and d) make their day more enjoyable. Each child was given an individualised progress chart on arrival, which was used throughout the day. The chart contained place markers resembling those found on a board-game, and children were able to place stickers onto these after the completion of tasks throughout the day (see Appendices 18 & 19 for training schedule and materials). Some tasks were additionally rewarded with confectionary, which children were able to take away with them in a 'goody bag'. The structured training session built up from having children lie still on the scanner bed for two minutes, through graded steps until they were eventually able to lie still inside the scanner and practice the two fMRI tasks, and be able to remain still for 10-15 minutes while wearing earplugs and headphones, and while watching a short cartoon. As well as minimising head movement during the scanning, these measures served to minimise anxiety, and none of the research participants expressed any concerns about the MRI scanning nor requested to withdraw from the study.

4.18 Participants

4.18.1 Recruitment

4.18.1.1 Initial recruitment

As mentioned above, the participants in the current study belonged to an existing cohort recruited at Imperial College London. Women were recruited over a 3-year period (starting December 2001) from patients awaiting amniocentesis at Queen Charlotte's Maternity Hospital, London. Maternal plasma and amniotic fluid samples were obtained from 267 women and later analysed for cortisol concentration (Sarkar, Bergman et al. 2007a; Sarkar, Bergman et al. 2007b). Mothers with full-term (≥37 weeks), healthy and singleton infants, with known birth outcomes, were invited to return to the hospital's paediatric clinic when their child was ~17 months old. For full details please see Bergman, Sarkar et al. (2007).

4.18.1.2 Current study recruitment

For the current study, I coordinated recruitment of participants with the assistance of a part-time Research Assistant (RA), who I trained and supervised. Mothers from the database of original participants (n=123) were sequentially approached and informed about the study. At the time of writing 35 mother pair dyads had been contacted. Eligibility criteria were: the child being

aged over 5½ when scanned, and being MR compatible. Children with neurological or neurodevelopmental disorders (e.g. autism spectrum disorder, attention deficit disorder, etc.) were not excluded from participating in the study as the focus of the study was to observe neural integrity across all children in order to assess the relative contribution of prenatal stress/*in utero* cortisol level.

Parents were initially contacted by sending a letter providing general study information, and notifying them I would make telephone contact soon to gauge their interest in participation (see Appendix 20 for initial contact letter). After approximately one week parents were contacted by telephone. Those who expressed interest were sent the Patient Information Sheet (see Appendix 21) by mail; this included a link to the video for them to watch with their child. After approximately 4 days they were recontacted by telephone and, if deciding to take part, were offered a convenient appointment date. As most parents requested dates falling within school holidays, scanning dates were limited and restricted the study's testing rate. Taxi transport was organised from either the participant's home or closest mainline station, to bring them to the Institute of Psychiatry.

Twenty parent-child pairs were recruited into the study to complete all parts (i.e. scanning and testing) out of the 35 parents contacted. Two further children were recruited into the study to undergo all testing excluding MRI. Reasons for this included one child being sensitive to noise – he had a diagnosis of Autistic Spectrum Disorder (ASD), and another who was considered unlikely to be able to lie still for the duration of scanning (n=1). Three parents withdrew from the

study and for the remainder no test dates were available at the time of writing (n=6). Of the 20 children who underwent scanning, useable neuroimaging data was acquired from 16 participants, with 4 datasets containing one or more unusable imaging sequences due to excess head movement (n=1), an inability to remain in the scanner (n=1), or from radiographers having been unable to acquire all imaging sequences within the allotted scanning slot (n=2).

Table 4.8: Breakdown of recruitment for Study 2

	Completed	Completed with no DT-MRI	Withdrew	Awaiting test date	Total contacted
Number of parent-child dyads	20	6	3	6	35

The entire cohort within this study comprised one group, who displayed variance on two dimensional indices of prenatal stress, namely: (i) number of maternal antenatal stressful life events in pregnancy: and (ii) prenatal amniotic fluid cortisol concentration.

Due to the nature of this study (preliminary, and based on limited participant availability) a power calculation to estimate sample size was not appropriate. Further, no prior study has used DT-MRI in a cohort of this type.

Group characteristics and all data pertaining to this group are given in Chapter 7.

4.19 Measures

4.19.1 Independent variables

Mothers and children in this study had undergone assessment at two time points prior to the start of the current study. Thus, I collected neuroimaging and behavioural data from the current (third) time point. However, data from the previous assessments constituted the major independent variables of the study; these data are outlined below.

4.19.1.1 Time point 1

The first time point at which participants were studied was at approximately 17 weeks antenatally (range 15-17 weeks), and the following measures were collected.

4.19.1.1.1 Maternal antenatal stress

4.19.1.1.1.1 Stressful life events

The primary predictor of antenatal stress was based on maternal report on the 26-item Stressful Life Events Questionnaire (Appendix 22) adapted from the Inventory of Ranked Life Events for Primiparous and Multiparous Women, which is a widely used index of stress in pregnancy (Barnett, Hanna et al. 1983). This inventory contained items measuring such diverse factors as illness, financial loss, and partner cruelty. For full details please see Bergman, Sarkar et al. (2007).

4.19.1.1.1.2 Amniotic fluid cortisol

The secondary antenatal stress predictor was the measurement of *in utero* cortisol, which was extracted from a small quantity (4ml) of surplus amniotic fluid taken from mothers at amniocentesis. For all mothers the amniocentesis was a routine procedure, and was being carried out mainly in order to karyotype for Down's syndrome. Cortisol was measured in nanomoles per litre (nM/L). For full details please see Sarkar, Bergman et al. (2007b).

4.19.1.1.2 Obstetric data

In addition to the two measures of antenatal stress, obstetric data was collected, including: maternal age, parity, ethnicity, smoking, alcohol and prescription drug use during pregnancy were recorded at recruitment. Neonatal data were also collected (birth weight, gestational age at birth, delivery method).

4.19.1.2 Time point 2

The second time point at which participants were assessed was at around 17 months postnatally (range 14-19 months), and the following assessments were conducted. Only variables of interest within Study 2 of the thesis are included.

4.19.1.2.1 Fear reactivity

Fear reactivity was assessed using the 'fear' subscale of the LabTAB (Laboratory Temperament Assessment Battery; Goldsmith and Rothbart 1999) test battery. This involved the presentation of a mechanical toy (robotic dog) that barked and opened and closed its mouth while moving unpredictably towards the infant. Reactions towards the toy were recorded, and a composite infant fearfulness score was given (see Bergman 2007 for additional details).

4.19.1.3 Time point 3

The third time point is the current stage during which I assessed participants; at this time point children were aged between 6 years old and 9 years old (see Table 14). The following parent-report questionnaires were administered to mothers:

Strengths and Difficulties Questionnaire (SDQ) – for the assessment of conduct problems, hyperactivity, emotional problems, and peer problems (for details see Study 1 - Chapter 5)

Antisocial Process Screening Device (APSD) – for the assessment of psychopathic/callous-unemotional traits (for details see Study 1 – Chapter 5).

4.19.2 Neuroimaging

At the current time point DT-MRI data were acquired using the same parameters as for Study 1 (see Chapter 5). In Study 2, the scanning session lasted for either sixty or ninety minutes, depending on availability of the scanner. This study presents data from only one of 13 MRI sequences that were run during the scanning session (i.e. the DT-MRI sequence); this was acquired towards the end of the scanning session. I performed tractography analysis on these data using the same tract of interest (uncinate fasciculus) and control tracts (inferior fronto-occipital fasciculus and inferior longitudinal fasciculus) as were examined in Study 1 (Chapter 5) and using the same methods as described above (see Tractography Analysis).

4.19.3 Statistics

I used SPSS to apply Spearman's bivariate analysis to examine correlations between the two measures of white matter microstructure of the uncinate fasciculus and control tracts (namely, fractional anisotropy, and perpendicular diffusivity) and (1) prenatal stressful life events; and (2) *in utero* cortisol concentration. This non-parametric test was selected for several reasons.

Firstly, it is more conservative than Pearson's r, its parametric equivalent (Dytham 1993). Secondly, several covariates have limited variability (e.g. antenatal stressful life events), which is dealt with better by Spearman's rho as this is based on ranked values. Finally, it has been suggested that DT-MRI data, such as FA, can not be regarded as having a normal distribution (Jones and Cercignani 2010). Further correlations examined the associate between DT-MRI measures and behavioural measures. Details of these analyses are given in Chapter 7.

4.19.4 Procedure

On arrival at the Institute of Psychiatry I greeted participants and showed them to the Centre for Neuroimaging Sciences. Here I (and the RA, who was present on many of the test days) gained full written, informed consent (see Appendix 23) from mothers (no fathers attended with their child) and were given the opportunity to ask questions. After this I ran the mock scanner training session, followed by participants being shown to the scanning suite, where they placed all loose and/or metallic objects into a locker. Children were given the opportunity to select a film to watch during their scan from a large selection of animated DVDs. After this, the duty radiographers took participants into the scanning room. Participants were given child-friendly sponge earplugs to protect from scanner noise, a pair of headphones through which to listen to their chosen film, and a two-button box with which to respond during the two functional tasks. Mothers were seated beside the scanner and were given the

emergency buzzer in case of their child expressing distress during the session; she was also given ear protection to minimise scanner noise.

A break was given for lunch, after which participants were taken to a child-friendly testing room adjoining a waiting area with age-appropriate toys for them to play with during breaks. Here, mothers were given questionnaires to complete while I administered neuropsychological tests, IQ testing, and two questionnaires to children. The children were given breaks between tasks during which sticker rewards and refreshments were offered. After completion of testing £50 in high street shopping vouchers was offered as reimbursement, and transport was provided. In accordance with the stipulation of the Ethics Committee, a letter was sent to the child's GP to inform them of their study participation.

The following three chapters of the thesis comprise three DT-MRI analyses using the methods described above.

5: Limbic-prefrontal ‘connectivity’ in Conduct disorder: a diffusion tensor imaging study

5.1 Abstract

Background: Children with Conduct disorder (CD) are at increased risk of developing antisocial personality disorder (ASPD) and psychopathy in adulthood. The biological basis for this is poorly understood. A preliminary diffusion tensor magnetic resonance imaging (DT-MRI) study of psychopathic antisocial adults reported significant differences from controls in the fractional anisotropy (FA) of the uncinate fasciculus (a white matter tract that connects amygdala to frontal lobe). However, it is unknown if developmental abnormalities are present in the uncinate fasciculus of younger individuals with CD.

Methods: I used DT-MRI tractography to investigate, for the first time, the microstructural integrity of the uncinate fasciculus in adolescents with CD, and age-related differences in this tract. I compared FA and perpendicular diffusivity of the uncinate fasciculus in 27 adolescents with CD and 16 healthy controls (12 to 19 years old) who did not differ significantly in age, IQ, or substance use history. To confirm that these findings were specific to the uncinate fasciculus, I extracted the same measurements from two non-limbic control tracts. Participants in the CD group had a history of serious aggressive and violent behaviour, including: robbery, burglary, grievous bodily harm, and sexual assault.

Results: Individuals with CD had a significantly increased FA (p=0.006), and reduced perpendicular diffusivity (p=0.002), in the left uncinate fasciculus. Furthermore, there were significant age-related between-group differences in perpendicular diffusivity of the same tract (Zobs= 2.40; p=0.01). Controls, but not those with CD, showed significant age-related maturation. There were no significant between group differences in any measure within the control tracts.

Conclusions: Adolescents with CD have significant differences in the 'connectivity' and maturation of uncinate fasciculus.

5.2 Introduction

The childhood antisocial behaviour disorder Conduct disorder (CD) is associated with increased risk for a variety of adverse outcomes including substance use disorders (Kessler, Nelson et al. 1996), mood disorders (Vloet, Konrad et al. 2008), adult Antisocial Personality Disorder (APSD) (Zoccolillo, Pickles et al. 1992a; Gelhorn, Sakai et al. 2007b), and psychopathy (Lynam, Caspi et al. 2007a). As outlined in the literature review (Chapter 1), neuroimaging studies of children with CD and/or callous-unemotional (CU) temperament have identified neural correlates of these phenotypes, and these mirror the deficits observed in antisocial and psychopathic adult samples. These findings predominantly localise to two brain regions: the limbic region/amygdala and the prefrontal/orbitofrontal cortex. For example, structural magnetic resonance imaging (sMRI) studies have identified reduced amygdala volume in both children (Sterzer, Stadler et al. 2007; Huebner, Vloet et al. 2008; Fairchild, Passamonti et al. 2011) and adults (Yang, Raine et al. 2009). Functional MRI (fMRI) has also found deficits in this structure during emotion processing tasks in antisocial children (Sterzer, Stadler et al. 2005; Herpertz, Huebner et al. 2008; Marsh, Finger et al. 2008a; Jones, Laurens et al. 2009; Passamonti, Fairchild et al. 2010) and criminal psychopaths (Kiehl, Smith et al. 2001; Birbaumer, Veit et al. 2005). Structural differences in the prefrontal/orbitofrontal cortex between healthy individuals and antisocial children (Huebner, Vloet et al. 2008; De Brito, Mechelli et al. 2009; Fahim, He et al. 2011) and adults (Raine, Lencz et al. 2000; Narayan, Narr et al. 2007; de

Oliveira-Souza, Hare et al. 2008) have also been found. Similarly, functional abnormalities have also been found in similar populations of antisocial children (Finger, Marsh et al. 2008; Marsh, Finger et al. 2008a; Rubia, Smith et al. 2009; Passamonti, Fairchild et al. 2010) and adults (Birbaumer, Veit et al. 2005). Finally, it has been suggested that CD beginning in childhood constitutes a distinct phenotype with stronger neurodevelopmental correlates than adolescence-onset CD (Moffitt 1993; Moffitt, Arseneault et al. 2008). However, recent neuroimaging studies show similar abnormalities in brain structure (Fairchild, Passamonti et al. 2011) and function (Passamonti, Fairchild et al. 2010) within both subtypes of CD.

Despite evidence for abnormalities within these two brain areas in antisocial populations it remains unclear to what extent each regional deficit contributes to, or is sufficient for giving rise to, these behavioural phenotypes. Furthermore, it is unlikely that CD can be fully explained simply by differences in the development of a single brain region; brain regions do not function in isolation, they form part of brain 'systems'. Hence it is crucial to also investigate the white matter connections linking brain regions putatively involved in CD. For example, there is initial evidence that adolescents with CD have reduced functional connectivity between the amygdala and OFC (Marsh, Finger et al. 2008b). However, little is known about the anatomy of limbic brain regions, or the connections between them, in children with CD. A preliminary study recently reported that antisocial adults with psychopathy had significant differences in fractional anisotropy (FA) of the uncinate fasciculus, a white matter tract connecting the amygdala and OFC (Craig, Catani et al. 2009),

suggesting reduced integrity of this tract in this population. FA value is derived from diffusion tensor MRI scanning (DT-MRI), and quantifies white matter microstructural integrity through measurement of longitudinal water molecule diffusion parallel to the axonal bundle. FA values range from 0 (perfectly isotropic diffusion) to 1 (perfectly anisotropic diffusion) (Pierpaoli and Basser 1996) - providing a proxy measure of tissue integrity (Horsfield and Jones 2002; Mori and Zhang 2006). The microstructural basis for FA value is thought to lie with properties such as the organisation within and between fibres, axonal calibre, and myelination (Beaulieu 2009; Paus 2010). In contrast, perpendicular diffusivity (D_{perp}), another DT-MRI derived value, measures diffusion occurring radially, across the fibre bundle. Increased D_{perp} occurs with demyelination, and is thus considered a marker for reduced membrane integrity that has its basis in reduced myelin content rather than in intra-axonal factors (see Beaulieu 2009). Further, reduced D_{perp} is seen in typical brain maturation and is associated with increasing FA (Lebel, Walker et al. 2008). For a more detailed description of DT-MRI please see Chapter 4.

In summary, there is preliminary evidence that microstructural integrity of the uncinate fasciculus (as measured using DT-MRI) is reduced in antisocial adults. It is not known whether a similar microstructural abnormality is evident in children who are at risk of ASPD or whether, as some authors have suggested, neural abnormalities observed in antisocial adults are confounded by the chronic exposure to alcohol and substance misuse typically found in these populations (Versace, Almeida et al. 2008). In order to address this issue, I recruited children with CD and a sample of healthy controls that did not differ

significantly on a range of socio-demographic variables, including level of hyperactivity, and substance use history. The main hypothesis was that children with CD would show deficits in the microstructural integrity of the uncinate fasciculus, as indexed by significant reduction in FA and increased D_{perp} compared to healthy controls. To confirm that any differences found were specific to the limbic amygdala–OFC network, the same measurements were extracted from two non-limbic control tracts; the inferior fronto-occipital fasciculus (IFOF) and the inferior longitudinal fasciculus (ILF). I also tested the subsidiary hypotheses that: 1) individuals with CD and controls have significant age-related differences in FA and D_{perp} of the uncinate fasciculus; and 2) the degree of white matter abnormality is related to severity of antisocial behaviour and callous-unemotional traits (CD/CU). In addition, as CD may be subdivided according to the presence of CU traits and the age of onset of the disorder, I conducted additional analyses to explore whether these distinctions are related to differences in white matter microstructure.

5.3 Materials and Methods

This study was approved by the Joint South London and Maudsley Research Ethics Committee (243/00). Full details of Materials and Methods are given in Chapter 4 of the thesis.

5.4 Participants

The study cohort comprised forty-three males aged between 12 and 19 years. Twenty-seven boys met a research diagnosis of CD, while 16 were healthy controls. There were no significant differences between groups in age, IQ, ethnicity, parental income, hyperactivity, and history of substance use (see Table 5.1).

All study participants: satisfied MRI safety requirements and were medication free, did not have a psychiatric history (other than CD, ADHD, or referrals for anger management), spoke English as their first language and were right handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). Only individuals with an IQ above 80 were included. In addition, the CD group was subdivided into those with high levels of callous-unemotional traits (CD+CU) and those without (CD-CU), as outlined previously in the Methods Chapter (see Chapter 4 for Recruitment Flowchart, Figure 4.3). Finally, the CD group was also subdivided into early-onset versus adolescence-onset boys to

examine the effects of age of onset on white matter microstructural integrity. See Table 5.5 for CD subgroupings.

5.5 Procedure

Full written informed consent was taken from participants, and additionally from a parent/guardian where boys were aged below 16 years old.

Table 5.1: Characteristics of Conduct disorder group and healthy controls

	CD (n = 27) Mean (SD)	Healthy controls (n=16) Mean (SD)	p value
Age in years	16 (2)	16 (2)	0.858
Mean FSIQ	99 (8)	103 (10)	0.098
Conduct problems (SDQ)	6 (2)	3 (1)	0.000**
Hyperactivity (SDQ)	7 (2)	6 (2)	0.375
Emotional Problems (SDQ)	4 (3)	3 (2)	0.061
Peer Problems (SDQ)	4 (2)	3 (2)	0.034*
Prosocial Behaviour (SDQ)	7 (2)	8 (1)	0.161
Total problems (SDQ)	18 (5)	12 (5)	0.000**
Callous-unemotional traits (APSD)	7 (2)	5 (2)	0.012*
Narcissism (APSD)	8 (3)	6 (3)	0.017*
Impulsivity (APSD)	7 (2)	6 (2)	0.048*
Total score (APSD)	25 (7)	19 (6)	0.005**
Boys with Child-onset CD	17	-	-
Boys with Adolescence-onset CD	10	-	-
Ethnicity (%)	n=27	n=16	Chi^2

White	52	63	0.717
Black/African-Caribbean	33	25	^0.735
Other	15	13	^1.000
Annual income (%)	n=17	n=10	Chi^2
Below £18,000	47	10	^0.091
£18,000-£25,000	24	40	^0.415
£25,000-£43,000	24	30	^1.000
Above £43,000	6	20	^0.535
Substance use (%)	n=20	n=15	Chi^2
Cannabis - ever used	60	40	0.407
Cannabis – used in past month	(n=12) 50	(n=6) 67	^0.638
Alcohol – ever used	75	100	^0.057
Alcohol – used in past month	(n=15) 73	(n=15) 67	^1.000
Cocaine – ever used	7	0	^0.496
Amphetamine – ever used	7	0	^0.496
[#]Any other drug – ever used	15	7	^0.619

*CD – Conduct disorder; FSIQ - Full Scale Intelligence Quotient; SDQ – Strengths and Difficulties Questionnaire; APSD – Antisocial Process Screening Device; SD – standard deviation; [#]Excluding alcohol and cannabis; ^Fishers exact probability test; *p<0.05; ** p<0.01*

5.5.1 DTI tractography:

Data were acquired and preprocessed as described in Chapter 4.

TrackVis software was used to hand dissect, in native space, the tract of interest (uncinate fasciculus), and the two control tracts (inferior fronto-occipital fasciculus, IFOF; inferior longitudinal fasciculus, ILF). I dissected these tracts in the same order for all data, and remained blind to clinical groupings throughout.

5.5.2 Statistical analysis

All statistical analyses were carried out using SPSS software (SPSS inc, Chicago, Illinois, USA. Repeated measures analysis was used with the within subjects variables of 'tract' (UF, IFOF, ILF) and 'hemisphere' (left, right); and 'group' (CD, controls) as the between subjects variable. This tested for significant differences in FA and D_{perp} between CD and control participants in the uncinate fasciculus and the two control tracts. *Post hoc* analyses were carried out to identify significantly differing tract values between boys with CD and healthy controls using one-way analysis of variance (ANOVA). Analyses were Bonferroni corrected for multiple comparisons.

Where a significant FA/D_{perp} difference was detected *post hoc* analyses were carried out to examine the relationship between DT-MRI measures and age in each group using Pearson's correlations; I then determined if there were

significant between group differences in these relationships using Z-observation analysis (Pallant 2007). Finally, I examined whether significant differences in DT-MRI parameters were associated with greater severity of conduct problems or callous-unemotional traits within, firstly, the whole sample and, secondly, the CD group only. Correlations were carried out between DT-MRI measures and: (i) total SDQ score; (ii) SDQ conduct problem score; (iii) total APSD score; and (iv) APSD callous-unemotional traits score, controlling for age.

These analyses were repeated within the CD group to explore differences between (i) those with (CD+CU) and without (CD-CU) high levels of CU traits, and (ii) those with early-onset (EO) versus adolescence-onset (AO) CD.

5.6 Results

The results are subdivided to examine, firstly, differences between CD and healthy controls (Analysis 1) and, secondly, between CD subtypes (Analysis 2).

5.6.1 Analysis 1 – Conduct disorder and healthy controls

5.6.1.1 Tractography

The CD group showed significant differences from controls in the left uncinate fasciculus, with a greater FA (CD 0.471; Control 0.451; p=0.006), and reduced D_{perp} (CD 0.583 x10-3 mm2/s; Control 0.611 x10-3 mm2/s; p=0.002). They also had significantly greater FA in the right uncinate fasciculus (CD 0.468; Control 0.455; p=0.040) - but this did not withstand Bonferroni correction. No significant differences were observed between groups in either FA or D_{perp} within the two control tracts (IFOF, ILF; Table 5.2; Figure 5.1). Further, there was no significant group-by-tract (F=1.07; p=.35), group-by-hemisphere (F=1.45; p=.24), or group-by-hemisphere-by-tract (F=0.97; p=.38) interaction.

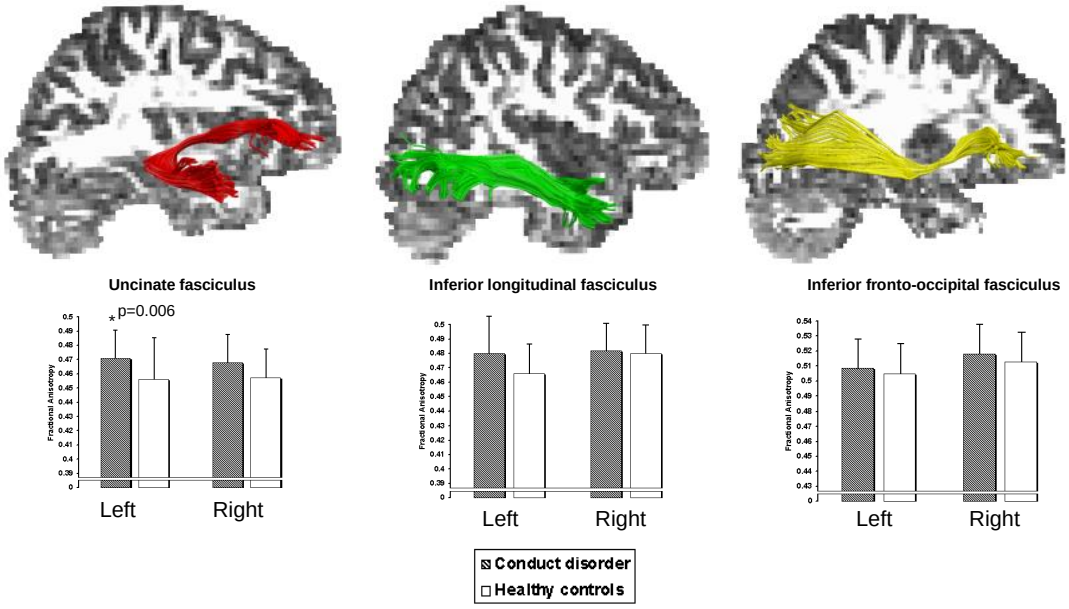
Table 5.2: DT-MRI measures of the uncinate fasciculus and control tracts in CD and healthy controls

Tract	Parameter	Hemisphere	Mean value CD (SD) n=27	Mean value Controls (SD) n=16	p value
Uncinate fasciculus	FA	Left	0.47 (0.02)	0.45 (0.02)	0.006*
		Right	0.47 (0.02)	0.46 (0.02)	0.040
	D_{perp}	Left	0. 58 (0.03)	0. 61 (0.03)	0.002*
		Right	0. 59 (0.03)	0. 60 (0.03)	0.306
Inferior fronto-occipital fasciculus	FA	Left	0.51 (0.02)	0.50 (0.02)	0.321
		Right	0.52 (0.02)	0.51 (0.02)	0.281
	D_{perp}	Left	0. 55 (0.03)	0. 57 (0.03)	0.115
		Right	0. 54 (0.04)	0. 56 (0.03)	0.159
Inferior longitudinal fasciculus	FA	Left	0.48 (0.03)	0.47 (0.02)	0.101
		Right	0.48 (0.02)	0.48 (0.02)	0.454

D_{perp}	Left	0. 57 (0.04)	0. 59 (0.03)	0.062
	Right	0. 57 (0.03)	0. 58 (0.04)	0.093

*SD – standard deviation; IFOF – inferior fronto-occipital fasciculus; ILF – inferior longitudinal fasciculus; FA – fractional anisotropy; D_{perp} – perpendicular diffusivity value and SD x10^{-3} mm^2/s; *significant after Bonferroni correction*

Figure 5.1: Between group differences in mean fractional anisotropy of the uncinate fasciculus and control tracts



5.6.1.2 Uncinate fasciculus and age

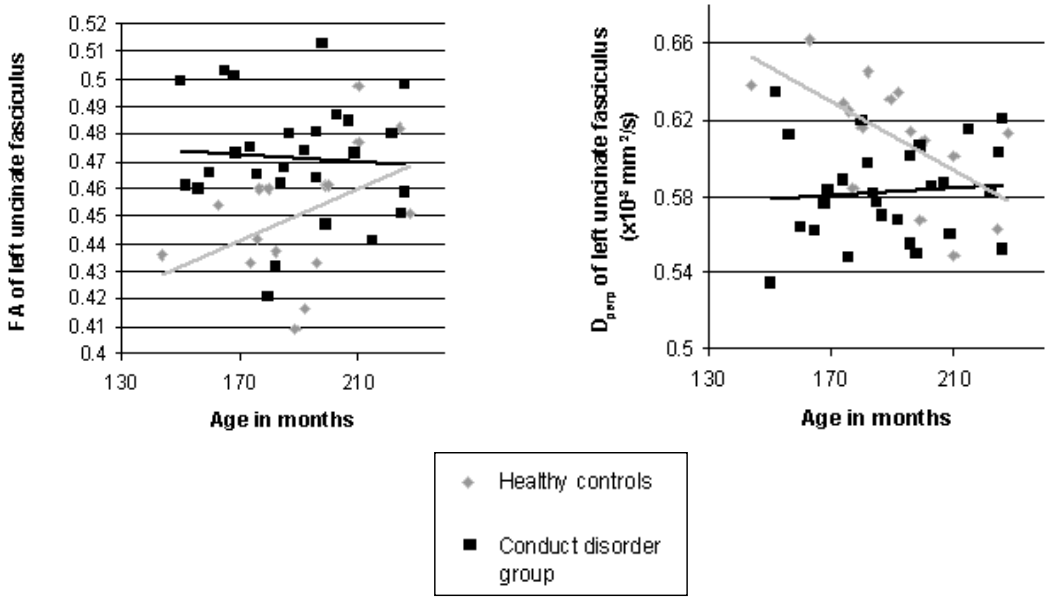
Within the CD group there were no significant correlations between left uncinate fasciculus FA or D_{perp} and age. In contrast, there was a significant negative correlation between age and D_{perp} of the left uncinate fasciculus (r=.-625; $p<0.05$) within the control group, and this differed significantly from the non-significant correlation seen in CD ($Z_{obs}=2.40$; $p<0.05$; Table 5.3, Figure 5.2).

Table 5.3: Pearson's correlations between left uncinate fasciculus FA and D_{perp} with age in CD group compared to healthy controls

	Age – CD group	Age – controls	Z_{obs} value
FA left (r/p)	-0.06/0.75	0.45/0.08	1.60
D_{perp} left (r/p)	0.09/0.64	-0.63/0.01*	2.40*

*FA – fractional anisotropy; r – correlation coefficient; p – p value; CD – conduct disorder; D_{perp} – perpendicular diffusivity; r- correlation coefficient; Z_{obs} – Z-observation; *two-tailed significance level - p<0.05*

Figure 5.2: Relationship between DT-MRI measures and age in the left uncinate fasciculus of adolescents with CD compared to healthy controls



FA – fractional anisotropy; D_{perp} – perpendicular diffusivity

5.6.1.3 Uncinate fasciculus and antisocial behaviour measures

Significant correlations were found between left uncinate fasciculus FA/D_{perp} abnormality and severity of all SDQ/APSD behavioural scores within the whole sample (Table 5.4). Although not statistically significant, there was a trend

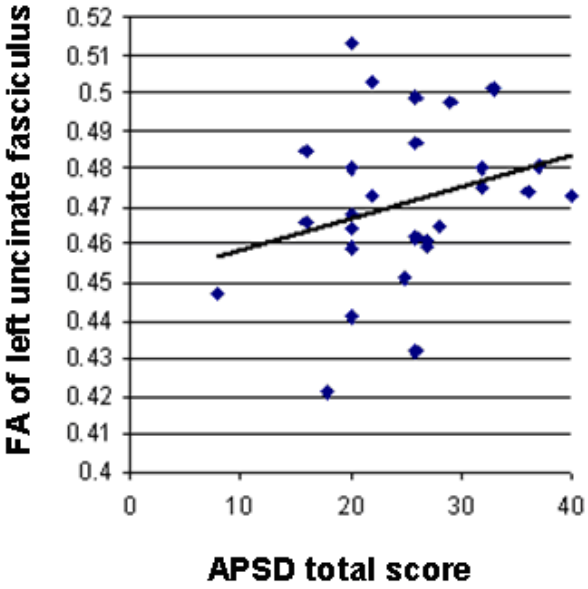
towards a positive correlation between total APSD score and age in the CD group alone (r=0.27; p = 0.09; Figure 5.3).

Table 5.4: Correlations between uncinate fasciculus DT-MRI measures and antisocial behaviour scores in whole sample

	Left uncinate fasciculus FA (r/p)	Right uncinate fasciculus FA (r/p)	Left uncinate fasciculus D_{perp} (r/p)
(i) SDQ total	0.32/0.019*	0.33/0.018*	-0.38/0.007**
(ii) SDQ CP	0.32/0.021*	0.27/0.042*	-0.37/0.008**
(iii) APSD total	0.40/0.005**	0.32/0.019*	-0.39/0.005**
(iv) APSD CU traits	0.40/0.004**	0.36/0.009**	-0.37/0.008**

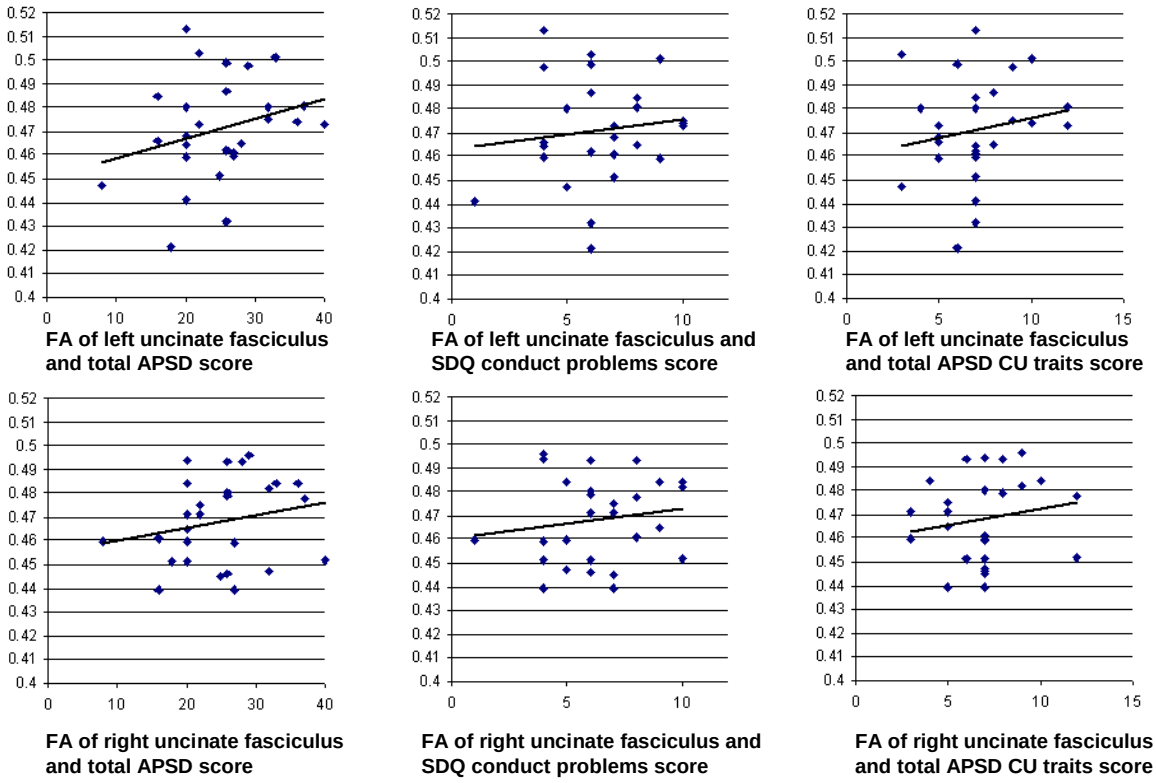
*SDQ – Strengths and Difficulties questionnaire; APSD – Antisocial Process Screening Device; CU – callous-unemotional traits; FA – fractional anisotropy; D_{perp} – perpendicular diffusivity; r – correlation coefficient; p – significance level; *p<0.05;**p<0.01*

Figure 5.3: Trend towards correlation between FA of the left uncinate fasciculus and total APSD score in CD group



FA – fractional anisotropy; APSD – Antisocial Process Screening Device; CD – Conduct disorder; r=0.271, p=0.09

Figure 5.4: Relationship between antisocial behaviour measures and uncinate fasciculus FA in Conduct disorder group



FA – fractional anisotropy; APSD – Antisocial Process Screening Device; SDQ – Strengths and Difficulties Questionnaire; CU – callous-unemotional

5.6.2 Analysis 2 – CU traits and age of onset in Conduct disorder

The first of two secondary analyses was carried out to determine whether indices of uncinate fasciculus integrity differed between individuals with CD with a high level of callous-unemotional traits and those without. Thus, individuals with CD were subdivided into two groups: CD+CU (CD with high a level of callous-unemotional traits) and CD-CU (CD without high levels of callous-unemotional traits), according to the grouping criteria outlined previously (see Chapter 4, Figure 4.3). Here CU traits is used in accordance with acceptable child taxonomy but is based on PCL-YV test scores, which classify 'psychopathic' traits; please see Chapter 1 for explanation of this issue.

Table 5.5: Characteristics of Conduct disorder subgroups

	CD+CU traits (n = 12) Mean (SD)	CD-CU traits (n=15) Mean (SD)	P value
Age	16 (2)	16 (2)	0.878
Mean FSIQ	102 (9)	96 (6)	0.074
Conduct problems (SDQ)	7 (2)	6 (2)	0.040*
Hyperactivity (SDQ)	8 (2)	7 (2)	0.235
Emotional Problems (SDQ)	3 (2)	4 (3)	0.391
Peer Problems (SDQ)	4 (2)	4 (2)	0.402
Prosocial Behaviour (SDQ)	7 (2)	8 (2)	0.655
Total Problems (SDQ)	19 (5)	18 (5)	0.487
Callous-unemotional traits (APSD)	9 (2)	6 (2)	0.001**
Narcissism (APSD)	10 (2)	7 (3)	0.002**
Impulsivity (APSD)	9 (1)	6 (2)	0.006**
Total Score (APSD)	31 (5)	20 (5)	0.000**

CD Threshold items (K-SADS-PL)	4 (2)	8 (2)	0.000**
Child-onset (%)	67	60	^1.000
Adolescence-onset (%)	33	40	^1.000
Ethnicity (%)	n=12	n=15	Chi^2
White	50	53	0.863
African-Caribbean	33	33	^1.000
Other	17	13	^1.000
Annual income (%)	N=7	N=10	Chi^2
Below £18,000	57	40	0.637
£18,000-£25,000	0	40	0.103
£25,000-£43,000	43	10	0.250
Above £43,000	0	10	^1.000
Substance use (%)	N=9	N=11	Chi^2
Cannabis - ever used	67	55	0.670
Cannabis – used in past month	(n=6) 50	(n=6) 50	^1.000
Alcohol – ever used	78	73	^1.000
Alcohol – used in past month	(n=7) 71	(n=8) 75	^1.000
Cocaine – ever used	22	0	0.189
Amphetamine – ever	22	0	0.189

used			
#Any other drug – ever used	11	9	^1.000

*CD+CU – Conduct disorder with high levels of callous-unemotional traits; CD-CU – Conduct disorder with low levels of callous-unemotional traits; FSIQ - Full Scale Intelligence Quotient; SDQ – Strengths and Difficulties Questionnaire; APSD – Antisocial Process Screening Device; SD – standard deviation; K-SADS-PL CD threshold items – Number of diagnostic items for Conduct disorder met at threshold on the Kiddie Schedule for Affective Disorders and Schizophrenia - Present and Lifetime; #Excluding alcohol and cannabis; ^Fishers exact probability test; *p<0.05; ** p<0.01*

It can be seen that the two subgroups differed significantly on the Conduct problems subscale of the SDQ, all ASPD subscales, and also in the number of items on the CD diagnostic interview (K-SADS-PL) that were met at threshold.

5.6.2.1 Tractography

There were no significant differences in FA or D_{perp} of the uncinate fasciculus or the two control tracts between CD boys with or without high levels of CU traits (Table 5.6).

Table 5.6: DT-MRI measures of the uncinate fasciculus and control tracts in CD with and without CU traits

Tract	Parameter	Hemisphere	Mean value CD+CU (SD) n=12	Mean value CD-CU (SD) n=15	p value
Uncinate fasciculus	FA	Left	0.47 (0.02)	0.47 (0.03)	0.445
		Right	0.47 (0.2)	0.47 (0.2)	0.604
	D_{perp}	Left	0.58 (0.02)	0.58 (0.03)	0.684
		Right	0.59 (0.03)	0.59 (0.03)	0.908
Inferior fronto-occipital fasciculus	FA	Left	0.50 (0.1)	0.51 (0.3)	0.170
		Right	0.52 (0.2)	0.52 (0.3)	0.617
	D_{perp}	Left	0.56 (0.02)	0.55 (0.03)	0.261
		Right	0.54 (0.02)	0.55 (0.04)	0.419
Inferior longitudinal fasciculus	FA	Left	0.48 (0.2)	0.48 (0.4)	0.791
		Right	0.48 (0.2)	0.49 (0.2)	0.458

D_{perp}	Left	0.57 (0.03)	0.57 (0.04)	0.730
	Right	0.57 (0.03)	0.56 (0.03)	0.695

CD+CU – Conduct disorder with high levels of callous-unemotional traits; CD-CU – Conduct disorder with low levels of callous-unemotional traits; SD – standard deviation; FA – fractional anisotropy; D_{perp} – perpendicular diffusivity (value and SD x10^{-3} mm^2/s)

There was no significant difference in DT-MRI measures between early-onset and adolescence-onset CD, although there was a trend towards lower D_{perp} of the left UF (Table 5.7).

Table 5.7: DT-MRI measures of uncinate fasciculus and control tracts in early-onset and adolescence-onset Conduct disorder

Tract	Parameter	Hemisphere	Mean value EO-CD (SD) N=17	Mean value AO-CD (SD) N=10	P value
Uncinate fasciculus	FA	Left	0.47 (0.02)	0.47 (0.02)	0.597
		Right	0.47 (0.02)	0.46 (0.02)	0.172
	D_{perp}	Left	0.58 (0.02)	0.59 (0.03)	0.080
		Right	0.59 (0.03)	0.60 (0.03)	0.149
Inferior fronto-occipital fasciculus	FA	Left	0.51 (0.02)	0.51 (0.03)	0.905
		Right	0.52 (0.02)	0.51 (0.03)	0.198
	D_{perp}	Left	0.55 (0.03)	0.56 (0.03)	0.563
		Right	0.54 (0.03)	0.55 (0.04)	0.289
Inferior longitudinal fasciculus	FA	Left	0.48 (0.03)	0.49 (0.03)	0.549
		Right	0.48 (0.02)	0.48 (0.02)	0.875

D_{perp}	Left	0.57 (0.04)	0.57 (0.03)	0.817
	Right	0.56 (0.03)	0.57 (0.04)	0.528

CD+CU – Conduct disorder with high levels of callous-unemotional traits; CD-CU – Conduct disorder with low levels of callous-unemotional traits; SD – standard deviation; FA – fractional anisotropy; D_{perp} – perpendicular diffusivity (value and SD x10^{-3} mm^2/s)

5.7 Discussion

This study compared the integrity of the uncinate fasciculus limbic tract between a group of boys with CD and a group of healthy controls. I found that adolescents with CD had significantly increased microstructural integrity of the uncinate fasciculus in comparison to healthy control boys. Further, these differences were tract specific, i.e. no differences were found in the non-limbic control tracts, plus there was no group by tract interaction, which further supports this specificity. *Post hoc* analysis showed that although D_{perp} declined with age in healthy children, it did not do so in the CD group. Further, the pattern of association with age was significantly different between the two groups. Moreover, *post hoc* analysis found a significant relationship between microstructural abnormality and severity of antisocial behaviour in the whole sample, although not in the CD group alone. These significant findings were found in the left uncinate fasciculus; however, there was a trend towards significance in the right hemisphere. Finally, I conducted secondary analyses to examine the effect of CU traits and age of onset of CD on white matter microstructure; these found no significant differences in DT-MRI indices between these two grouping parameters.

These results support the *a priori* hypothesis that antisocial behaviour is associated with specific abnormalities in limbic connections (i.e. as opposed to global white matter changes). However, this abnormality was in the opposite direction to that predicted (namely an increase rather than a decrease in FA –

discussed below). The uncinate fasciculus tract is the major fronto-temporal limbic tract, and it connects the amygdala and orbitofrontal cortex. Damage to this tract leads to impairments of conditional associative learning in animals (Gaffan and Eacott 1995; Gutnikov, Ma et al. 1997). Reversal learning - a form of conditional associative learning – involves learning to 'reverse' responses that were previously rewarded but are later punished. Difficulties with reversal learning have been demonstrated in adults with antisocial behaviour and psychopathy (Budhani, Blair et al. 2005; Budhani, Richell et al. 2006). Further, children with CD and CU traits show abnormal BOLD activation in ventromedial prefrontal cortex on reversal tasks during functional magnetic resonance imaging (Finger, Marsh et al. 2008). It has been suggested that reversal learning deficits contribute to the perseveration of antisocial behaviour in both young people and adults, whereby individuals fail to learn to avoid behaviour that has negative consequences for themselves and others (Sundram, Deeley et al. 2012). My findings may help explain the biological basis of that, and that should be a focus of future studies.

Similarly, altered 'connectivity' between the orbitofrontal cortex and amygdala secondary to abnormal development of the uncinate fasciculus may contribute to impaired regulation of amygdala activity by the orbitofrontal cortex, which in turn may contribute to the abnormal emotional processing and behavioural disinhibition encountered in young people with conduct disorder and adults with ASPD/psychopathy (Sarkar, Clark et al. 2011). For example, a prior study reported reduced functional 'connectivity' between the amygdala and ventromedial prefrontal cortex in children with CD and CU traits during an

emotional processing task (Marsh, Finger et al. 2008a). Further, a DTI study of healthy children reported that a measure of engagement in dangerous and risky activities correlated positively with FA, and negatively with D_{perp}, in frontal white matter tracts (Berns, Moore et al. 2009). Also, these authors proposed that this may be attributable to earlier maturation of these tracts in high scoring youngsters. Together these studies support the view that abnormal fronto-temporal 'connectivity' may underlie the difficulties in behavioural and/or emotional regulation observed in antisocial children.

In addition, these findings raise the question of how age, behaviour, and disorder related differences in DT-MRI measures relate to underlying biology and its developmental determinants. My results suggest that while in typical children D_{perp} of the left uncinate fasciculus decreases with age, in CD it does not. Moreover, the relationship between this DT-MRI measure and increasing age significantly differed between groups. As discussed previously, myelination is one process thought to underlie the decreasing D_{perp} values and increasing FA seen in typical maturation (Song, Sun et al. 2003; Lebel, Walker et al. 2008), alongside greater axonal calibre (Paus 2010) and reduced neuronal branching (Silk, Vance et al. 2009). I found significantly reduced D_{perp} in the left uncinate fasciculus accompanied by increased FA in CD, suggesting that these individuals may differ from controls with respect to the myelination of this tract. It is known that myelination differs by age and brain region (Lebel, Walker et al. 2008), and is modulated by learning and environmental experience. For example, increased myelination has been found to accompany intensive practice of motor tasks, such as piano playing (Bengetsson, Nagy et al. 2005)

and juggling (Scholz, Klein et al. 2009). Also, children who experience severe deprivation in early childhood have significant differences in FA of the left uncinate fasciculus as compared to control children (Eluvathingal, Chugani et al. 2006). Similarly, it was reported that young adults exposed to high levels of verbal abuse from their parents during childhood have significant differences in FA of two left hemisphere limbic tracts (cingulum and fornix) and the arcuate fasciculus (Choi, Jeong et al. 2009). Therefore, differences in tract integrity seen in developmental psychopathologies, including my study, may have arisen from a complex mixture of social and biological factors. This highlights both the need for future studies of white matter maturation to consider (for example) environmental and social variables, and the potential relevance of early interventions to prevent or moderate the course of developmental disorders.

The *post hoc* investigation of behavioural measures revealed a significant association between CD/CU traits and uncinate fasciculus FA/D_{perp} in the sample as a whole, but only a trend to significance in the CD group alone. This preliminary finding suggests that this tract may contribute towards the generation of behavioural variation in adolescents, perhaps through an increased 'connectivity' between frontal and limbic systems; but further (larger) studies are required.

Importantly, the FA abnormality I found in CD adolescents was in the opposite direction to that previously reported in adults with antisocial behaviour (i.e. increased rather than decreased) (Craig, Catani et al. 2009), that my hypothesis was based on. One possible explanation for this may be that white matter

maturation of the uncinate fasciculus in children with CD follows a different developmental trajectory to that of healthy individuals - consistent with abnormal patterns of white matter maturation in non-CD children who indulge in extreme risk taking behaviours (Berns, Moore et al. 2009). This could indicate an initially accelerated rate of white matter microstructural development in adolescents with CD that fails to progress, or possibly even declines, in adulthood relative to typical development. Therefore, the reduced FA found in Craig et al's adult study may simply be the progression of this childhood abnormality. Such a mechanism may contribute towards a neurobiological explanation for the high rates of recidivism and treatment resistance found within persistently antisocial populations. For example, abnormal/precocious white matter maturation in children with CD may interfere with neural mechanisms underpinning pro-social behaviour. However, it is only possible to investigate such a hypothesis through future longitudinal studies.

Finally, with regard to the secondary analyses that examined the influence of CU traits and age of onset on white matter microstructure these each showed no difference between CD subtypes. There was a trend towards reduced D_{perp} of the left uncinate in the left uncinate in early-onset CD that was not seen in the adolescence-onset group. It has been suggested that conduct problems that begin in childhood (below age 10) have a greater neurodevelopmental basis, whereas later onset is more the result of peer influences than neurobiology (Moffitt 1993; Moffitt, Arseneault et al. 2008). Previous structural neuroimaging studies of CD have bolstered this idea by examining only early-onset cohorts (Kruesi, Casanova et al. 2004; Sterzer, Stadler et al. 2007; Huebner, Vloet et al.

2008). However, recent studies have found both child- and adolescence-onset CD subtypes to show similar neural abnormalities (Passamonti, Fairchild et al. 2010; Fairchild, Passamonti et al. 2011). Thus, my findings support this evidence that neuroanatomical abnormalities are present in all conduct disordered children irrespective of age of onset.

5.8 Limitations

This study has several limitations. Firstly, it did not find significant correlations between uncinate fasciculus abnormality and antisocial behaviour measures, and this may have resulted from of a lack of power due to small sample size. Furthermore, a larger sample size may have revealed significant differences between white matter measures of boys with high levels versus low levels of CU traits. This may have then revealed correlations between DTI measures and behavioural scores within the CD+CU group, enabling an examination of how these related to PCL-YV total and factor scores. This could have been of potential interest as children with higher CU traits and PCL-YV scores are at increased risk for more severe and chronic antisocial behaviour (Frick and Marsee 2006). Similarly, early-onset CD is similarly associated with greater levels of delinquency, aggression and violence (Kazdin 1995; Jeglum Bartusch, Lynam et al. 1997) than a later onset. However, in this study age of onset was not associated with increased anatomical abnormality. Whilst this finding is consistent with recent studies (Passamonti, Fairchild et al. 2010; Fairchild,

Passamonti et al. 2011), future studies would benefit from examining this issue within larger samples.

Secondly, in this study I recruited antisocial children with CD from predominantly non-forensic community samples. If I had recruited from juvenile detention centres, and other forensic settings, I would probably have identified children with more severe antisocial behaviour. This in turn may have highlighted greater differences in white matter measures between groups that may have associated with behavioural measures. However, given the difficulty of recruiting from such settings the current study selected participants with the highest levels of antisocial behaviour that could be found in community samples. Further, these findings are more generalisable to the wider population of children with CD, whose antisocial behaviour is significant whilst not generally crossing the threshold for incarceration within the criminal justice system. Conversely, one of the strengths of the study lies in its recruitment of a healthy control group who closely resemble the CD group in many respects, such as IQ, hyperactivity, and other socio-demographic factors. This helps to address some research issues surrounding, for example, the confounding effects of drug and alcohol use, which is commonplace among antisocial samples. One final caveat is that two of the four behavioural measures have a recommended upper age limit of 16 and 17, namely the APSD and SDQ, respectively. However, as these were used only as screening measures prior to grouping participants using the K-SADS-PL and PCL-YV, this was unlikely to affect the results.

5.9 Conclusion

In summary, this study showed that adolescents with CD significantly differ from controls in the microstructural anatomy, and possibly the maturation, of the uncinate fasciculus. While only a longitudinal design can confirm age effects, these findings suggest that the microstructural development of the uncinate fasciculus follows an altered course in boys with CD. However, it is not clear how these abnormalities arise, or whether they predict outcome. Studies of antisocial behaviour within larger cohorts, and across the lifespan, are required.

6: A whole-brain investigation of white matter microstructure in adolescents with Conduct disorder

6.1 Abstract

Background: As described in Chapter 2, adolescents with Conduct disorder (CD) have significant differences in grey matter anatomy and function of several brain regions as compared to healthy controls. However, brain regions do not function in isolation, and childhood antisocial phenotypes are likely to arise from the combination of activity and function occurring *between* interconnected brain regions. In particular, abnormality of the major tract within the limbic-prefrontal network - the uncinate fasciculus (UF) - is associated with antisocial behaviour in adults (Craig, Catani et al. 2009). Therefore, in Chapter 5 of this thesis I used DT-MRI tractography to examine the *a priori* hypothesis that boys with CD have abnormal microstructural integrity of the UF as compared to healthy controls, and found increased integrity in CD. My results suggested that increased UF integrity may reflect accelerated white matter maturation in CD. However, that study examined only a predefined region of interest (the UF, plus two further comparison tracts), and not whole brain (i.e. it is unclear how specific that finding was). Therefore, I carried out a further study using a whole-brain DT-MRI approach in youngsters with and without CD to explore whether other WM tracts also have increased microstructural integrity. Tract-based spatial statistics (TBSS) is an automated method of whole-brain voxel based white matter analysis. To date no study has examined white matter in CD using TBSS; or related differences in microstructural integrity to behaviour and/or age.

Methods: I compared whole brain white matter fractional anisotropy (FA) using TBSS from DT-MRI scans of 27 adolescents with CD and 16 healthy controls aged between 12 and 19 years old, who did not significantly differ in age and IQ. Further analyses examined relationships between (i) FA and behavioural measures; and (ii) FA and age.

Results: The CD group, compared to controls, had clusters of significantly ($p<0.05$; corrected for multiple comparisons) greater FA in 8 brain regions corresponding to: 1) the bilateral inferior and superior cerebellar peduncles, corticopontocerebellar tract, posterior limb of internal capsule, and corticospinal tract; 2) right anterior thalamic projection and superior longitudinal fasciculus; and 3) left cerebellar white matter. Severity of CD/CU symptoms were significantly correlated with FA in several of these regions in the total sample, but not in the CD or control groups alone. Further, typically developing adolescents showed significant age-related increases in FA in 3 regions: the bilateral middle and superior cerebellar peduncles, and bilateral cortico-spinal tract. In contrast, no significant age-related increases in FA were found in the CD group. However, the relationship of FA with age did not significantly differ between groups.

Conclusion: Adolescent boys with CD have significantly greater FA than controls in areas corresponding to white matter tracts within the fronto-cerebellar brain circuit, which connects the prefrontal cortex and cerebellar regions to the brainstem and spinal cord. These tracts are known to be

involved in aggression and affective processing, as well as being integral to motor function; and I found the first (albeit preliminary) evidence that variation in their microstructure may be dimensionally related to behaviour problems in youngsters. Finally, higher FA in most of these same regions was seen with increasing age in controls, but not in CD, suggesting anomalous white matter maturation in CD compared to typically developing boys. In summary, these findings are consistent with the hypothesis that antisocial behaviour in young people is associated with abnormalities in white matter 'connectivity'.

6.2 Introduction

As described in Chapter 2, attempts to identify the neurobiological bases of CD have revealed abnormalities within the grey matter of temporo-limbic and prefrontal brain regions (Kruesi, Casanova et al. 2004; Sterzer, Stadler et al. 2007; Huebner, Vloet et al. 2008; De Brito, Mechelli et al. 2009; Fahim, He et al. 2011; Fairchild, Passamonti et al. 2011). Functional imaging studies have also reported regional differences in activation of these same regions (Sterzer, Stadler et al. 2005; Herpertz, Huebner et al. 2008; Marsh, Finger et al. 2008a; Jones, Laurens et al. 2009; Rubia, Smith et al. 2009; Passamonti, Fairchild et al. 2010). Hence there is increasing evidence that specific brain regions are implicated in CD. However, brain regions do not act in isolation – they form part of large scale neural *networks*. Thus it is important to also examine the 'connectivity' of particular neural systems. There is preliminary evidence that antisocial behaviour may arise as a result of functional differences in the limbic-prefrontal network (that is associated with the generation of complex social and emotional behaviours) (Marsh, Finger et al. 2008a; Decety, Michalska et al. 2009; Rubia, Smith et al. 2009). The anatomical substrate for these functional differences is unknown – but recent studies of antisocial adults suggest that it may include deficits in the uncinate fasciculus (UF; a white matter tract that forms an essential part of the limbic-prefrontal circuit) (Craig, Catani et al. 2009; Motzkin, Newman et al. 2011; Sundram, Deeley et al. 2011). No previous study, however, has used DT-MRI to examine the microstructural integrity of this tract in CD. Therefore, in the previous chapter (Chapter 5) of this thesis I

used DT-MRI tractography to test the *a priori* hypothesis that microstructural integrity (as indexed by FA; fractional anisotropy) of the UF would differ significantly between adolescents with CD and healthy controls. I found that in CD youngsters FA of this tract was significantly increased as compared to controls, but the FA of the two non-limbic control tracts was not. Therefore, I hypothesised that adolescents with CD may have specific abnormalities in development of the limbic-prefrontal network.

Therefore, in order to explore the regional specificity of my finding (i.e. whether any additional tracts show abnormal microstructural integrity in CD) I then carried out a study using a whole-brain DT-MRI approach. Tract-based spatial statistics (TBSS) is a whole-brain voxel-based approach to the investigation of white matter integrity (Smith, Jenkinson et al. 2006) as indexed by fractional anisotropy (FA) (see Materials and Methods – Chapter 4 for full details). I compared FA across the whole brain in boys with CD and healthy controls.

6.3 Methods and Materials

This study was approved by the Joint South London and Maudsley Research Ethics Committee (243/00). For full details of methods please see Chapter 4.

6.3.1 Participants

This study used the same cohort as that reported in Chapter 5. Namely, 27 right handed males aged between 12 and 19 years with CD, and sixteen healthy right-handed controls with no significant difference in age, substance use history and IQ (see Table 5.1, Chapter 5).

All study participants satisfied MRI safety requirements, were medication free, spoke English as their first language and were right handed as assessed by the Edinburgh Handedness Inventory (Oldfield, 1971). Full scale IQ (FSIQ) was measured using the vocabulary and matrix reasoning subtests of the Wechsler Abbreviated Scale of Intelligence (Psychological Corporation, 1999). Only participants with an IQ above 80 were included.

6.3.2 DT-MRI analysis and statistics

The FA maps generated from each participant's raw DT-MRI data in the previous study, detailed in Chapter 4, were used in this analysis. First, the FA maps were transformed into standard stereotactic space using a study specific template generated in FSL (fmrib Software Library, www.fmrib.ox.ac.uk/fsl), which was the FA map most representative of all FA images within the sample. All FA maps were averaged into a mean FA map for the whole sample and an average skeleton was created onto which each participant's aligned FA data was projected. Finally, TBSS (part of FSL (Smith, Jenkinson et al. 2004)) was applied to diffusion data for voxelwise analysis of whole brain white matter (Smith, Jenkinson et al. 2006). Age and FSIQ, by group, were included as covariates in the design matrices used in the analysis, and results were corrected for multiple comparisons. The relevant contrasts were: CD>Controls; Controls>CD; Age CD+; and Age Controls+. Regions showing significant FA differences (with a threshold of $p<0.05$; corrected for multiple comparisons) between groups were identified with reference to WM atlases (Catani and Thiebaut de Schotten 2008). For full details of TBSS analysis please see Chapter 4. For later correlation analysis (with behavioural and age data) it was necessary to extract FA values from regions of interest using masks created in FSL using the JHU white matter atlas (Mori, Wakana et al. 2005), and using the 'fslmeants' function. Where bilateral differences in FA were found within a region, composite masks of left and right hemisphere tracts were made. FA values were correlated with SDQ and APSD behavioural scores, and age, using

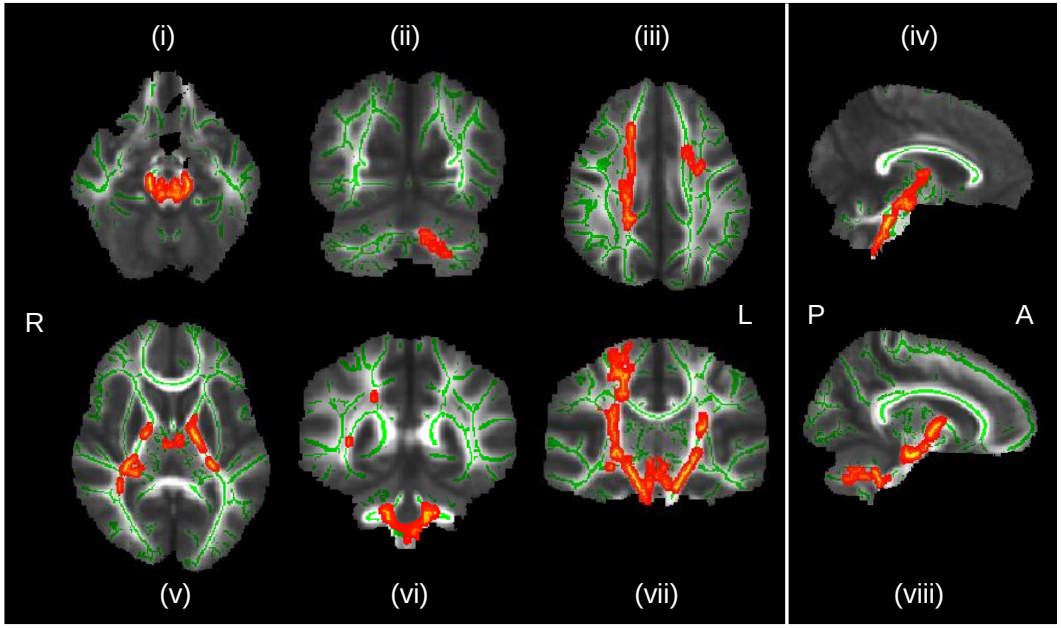
Spearman's rho non-parametric correlation. Finally, Z-observation (Z_{obs}) analysis was used to examine whether correlations between FA and age differed significantly between the CD group and healthy controls (Pallant 2007).

6.4 Results

6.4.1 Between group analysis

The CD group had significantly greater FA as compared to healthy controls in 8 regions: 1) bilaterally in the inferior and superior cerebellar peduncles, corticopontocerebellar tract, posterior limb of internal capsule, and corticospinal tract; 2) in the right - anterior thalamic projection and superior longitudinal fasciculus (SLF); and 3) in left - cerebellar white matter (see Figure 6.1). Controls had no areas with significantly greater FA.

Figure 6.1: Regions of significantly greater fractional anisotropy in conduct disordered adolescents compared to healthy controls (p<0.05 corrected for multiple comparisons)



Key: *R – light; L – left; A – anterior; P – posterior; green indicates mean FA (fractional anisotropy skeleton); red denotes areas of significantly greater (p<0.05) FA in CD in: (i) bilateral superior cerebellar peduncle; (ii) left cerebellar white matter; (iii) right superior longitudinal fasciculus; (iv) bilateral corticopontocerebellar tract; (v) bilateral posterior limb of internal capsule; (vi) bilateral inferior cerebellar peduncle; (vii) bilateral corticospinal tract; right anterior thalamic radiation (viii) bilateral corticopontocerebellar tract*

6.4.2 Behavioural analysis

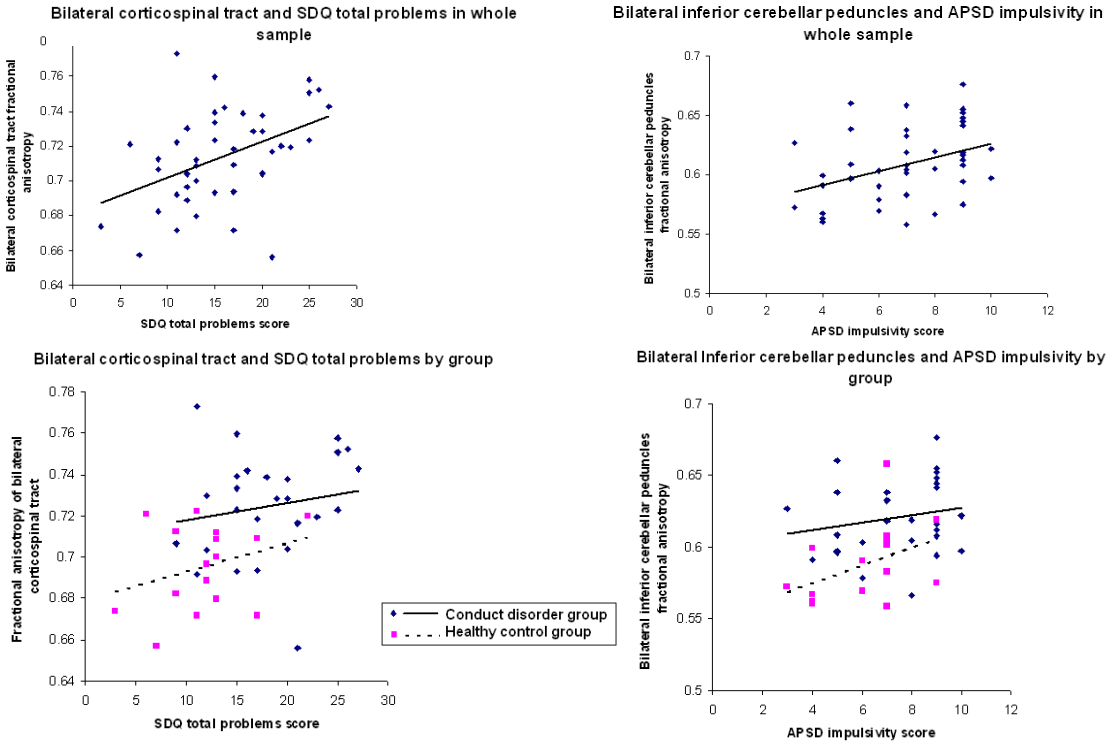
SDQ and APSD scores were positively correlated with FA values in 4 of the 8 regions that showed between group differences in the whole sample, but not within the CD or healthy control groups. There were significant positive correlations between: 1) SDQ total problems and FA of the corticospinal tract (0.423; p=0.005), SLF (r=0.345; p=0.023), and inferior cerebellar peduncles (r=0.378; p=0.012); 2) SDQ conduct problems and FA of the corticospinal tract (r=0.406; p=0.007), internal capsule (r=0.361; p=0.017), and inferior cerebellar peduncles r=0.439; p=0.003); 3) APSD total problems and the inferior cerebellar peduncles (r=0.349; p=0.022); and 4) APSD impulsivity and the SLF (r=0.302; p=0.049) and inferior cerebellar peduncle (r=0.401; p=0.008) (see Table 6.1) Figure 6.2 shows a sample of the correlations between FA of the above regions and behaviour scores in both the whole sample (significant correlations) and also within each of the groups (non-significant correlations). The latter are included to illustrate data distributions.

Table 6.1: Correlations between SDQ and APSD scores and fractional anisotropy in whole sample

Tract region	SDQ total problems		SDQ Conduct problems		APSD total problems		APSD CU traits		APSD Impulsivity		APSD Narcissism	
	r	*P*	*r*	*p*	*r*	*p*	*R*	*p*	*r*	*p*	*r*	*p*
Bilateral corticospinal tract	.423	.005**	.406	.007**	.209	.178	.206	.185	.124	.427	.200	.200
Bilateral posterior limb of Internal capsule	.287	.062	.361	.017*	.146	.350	.249	.107	.215	.165	.037	.813
Bilateral inferior cerebellar peduncle	.378	.012*	.439	.003**	.349	.022*	.296	.054	.401	.008**	.218	.160
Right superior longitudinal fasciculus	.345	.023*	.190	.222	.289	.060	.233	.133	.302	.049*	.242	.118

*SDQ – Strengths and Difficulties Questionnaire; APSD – Antisocial Process Screening Device; r – Spearman's correlation coefficient; p– two-tailed significance level; *p<0.05; **p<0.01*

Figure 6.2: Correlations between FA and behavioural measures in whole sample (above) and by group (below)

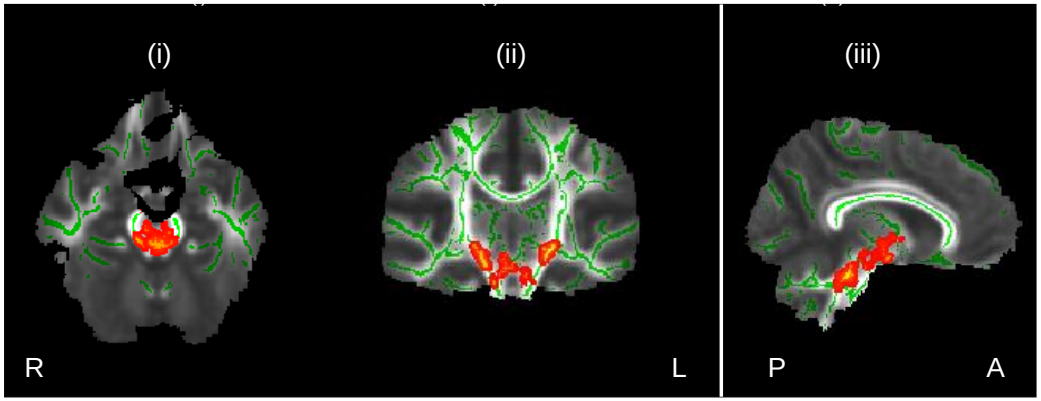


SDQ – Strengths and Difficulties Questionnaire; APSD – Antisocial Process Screening Device; top plots show significant correlations between tract regions and behavioural scores in the whole sample; lower plots show the non-significant correlations of the same data by group.

6.4.3 Age related analysis

The control group showed significantly increased FA with age in 3 regions: bilateral middle and superior cerebellar peduncles, and cortico-spinal tract (see Figure 6.3; Table 6.2). No tracts showed a significant relationship between FA and age in the CD group.

Figure 6.3: Regions showing significant positive correlations between fractional anisotropy and age in healthy control adolescents (p<0.05 corrected for multiple comparisons)



Key: *R – light; L – left; A – anterior; P – posterior; green indicates the mean FA (fractional anisotropy) skeleton; red denotes areas showing a significant positive correlation (p<0.05) between FA and increasing age in: (i) bilateral middle cerebellar peduncle; (ii) bilateral corticospinal tract; bilateral superior cerebellar peduncle (iii) bilateral corticospinal tract*

Table 6.2: Correlations between fractional anisotropy and increasing age

Bilateral white matter region	Conduct disorder		Healthy controls	
	R	*p*	*r*	*p*
Corticospinal tract	0.306	0.121	0.631	0.009**
Middle cerebellar peduncle	0.229	0.250	0.537	0.032*
Superior cerebellar peduncle	-0.272	0.169	0.567	0.022*

*r – Spearman's correlation coefficient; p– two-tailed significance level; *p<0.05; **p<0.01*

Z_{obs} analysis showed that the relationship between FA and age did not significantly differ between the CD and control group (Table 6.3).

Table 6.3: Results of Z-observation analysis comparing the relationship between FA and age in each group

Bilateral white matter region	Z_{obs}
Corticospinal tract	-1.253
Middle cerebellar peduncle	-1.130
Superior cerebellar peduncle	-1.186

Z_{obs} – Z-observation analysis score: non-significant values are those falling between -1.96 and 1.96.

6.5 Discussion

This study used whole-brain voxel-based DT-MRI to explore white matter microstructural integrity within a sample of adolescent boys with CD and a healthy comparison group. There was significantly increased FA within regions corresponding with the trajectories of several white matter tracts in CD boys compared to healthy controls, namely: 1) bilaterally in the inferior and superior cerebellar peduncles, corticospinal tract, internal capsule (posterior limb), and corticopontocerebellar tract; 2) in the right superior longitudinal fasciculus and anterior thalamic radiation; and 3) left cerebellar white matter. There were no areas of significantly reduced FA in CD as compared to controls. Correlation analysis found significant positive associations between FA of several tracts and behavioural variables in the total sample, but not in the CD or control groups. The correlations were between: 1) SDQ conduct problems and FA of the corticospinal tract, internal capsule and inferior cerebellar peduncles; 2) SDQ total problems and FA of the corticospinal tract, SLF and inferior cerebellar peduncles; 3) APSD total problems and the inferior cerebellar peduncles; and 4) APSD impulsivity and the SLF and inferior cerebellar peduncle. A further analysis found that the control group, but not the CD group, showed a significant positive correlation between FA and age in a number of regions corresponding to: 1) bilateral middle cerebellar peduncles; 2) bilateral superior cerebellar peduncles; and 3) bilateral corticospinal tract. However, the relationship between FA and age in these regions did not significantly differ between groups.

While the precise significance of FA is not agreed on, it is regarded as a measure of inter- and intra-axonal properties, including the organisation within and between fibres, axonal diameter, and myelination (Beaulieu 2009; Paus 2010). Therefore, the increased FA reported here may reflect differences in white matter organisation or greater myelination of axonal tracts in CD. In healthy children and adolescents FA increases with age; this corresponds to white matter microstructural changes that occur between infancy and early adulthood (Lebel, Walker et al. 2008; Marsh, Gerber et al. 2008). In my study, I found that FA was not correlated with age in CD, whereas it increased in healthy controls with aging. Thus, the increased FA observed in this study may result from an early (accelerated) rate of white matter maturation (e.g. myelination) in CD. Moreover, the between-group differences in FA may be a general indication of abnormal neurodevelopment. However, the relationships between FA and age did not differ significantly between groups. It is possible that this is due to small samples size. Thus, future studies should use larger samples to examine this further and, preferably, examine the same children at different time points as part of a longitudinal study.

The increased FA seen in boys with CD in this study was observed in a number of brain areas. While TBSS identifies clusters of significantly different FA between groups, it does not identify individual tracts *per se* (i.e. it identifies brain regions that contain tracts, but not the tracts themselves). Nevertheless, I identified several significant clusters falling along the length of tract trajectories

(i.e. they are not only single points within a tract). The trajectories of these clusters correspond predominantly to projection tracts, which connect cortical and subcortical regions, including: the corticospinal and corticopontocerebellar tracts, the thalamic radiation, and the posterior limb of the internal capsule (which carries the corticospinal tract fibres (Paus, Zijdenbos et al. 1999)). These white matter tracts connect the cerebral cortex with the brainstem, thalamic nuclei, and the pons (which is further connected to the cerebellum). The pons is the bridge between the cerebellum and the rest of the brain, and relays sensory signals between these areas. As well as its role in sleep and respiration, functions of the pons include sensory analysis, motor control, and the production of facial expressions. Animal studies have shown that stimulation to the pons can provoke predatory attack (Berntson 1973). This may explain how significant differences in white matter integrity (which could cause differences in signal conduction through this region) may give rise to aggressive or antisocial behaviour in CD youngsters.

I also found between group differences in FA in the superior and inferior cerebellar peduncles. These fibre bundles are composed of efferent projections to the thalamus (superior cerebellar peduncles) or afferent projections from the spinal cord (inferior cerebellar peduncles). The major input to the cerebellar peduncles originates from the prefrontal, as opposed to the motor, cortex (PFC) (Ramnani, Behrens et al. 2006), and this input is received in the most part via the corticopontine tract (Tomasch 1969). Thus, FA differences in these areas may indicate abnormal integrity of the white matter circuit connecting the PFC to

the pons and cerebellum in boys with CD. Further, this may stem from abnormality originating from input provided by the PFC – as a number of prior neuroimaging studies have reported that CD individuals have significant differences in the anatomy and function of this brain region (e.g. De Brito et al. 2009; Finger et al. 2008). However, the current data cannot determine whether abnormality of the PFC is primary or secondary to differences in this tract. Longitudinal studies are required to elucidate this issue.

Evidence that increased FA of the cerebellar peduncles may be relevant to the generation of antisocial behaviour comes from patients with damage to PFC-cerebellar circuits. These individuals display some of the same deficits observed in antisocial children and adults. (i.e. emotional processing difficulties (Schmahmann and Sherman 1998; Turner, Paradiso et al. 2007; Stoodley and Schmahmann 2009) and deficits of conditional associative learning (Sacchetti, Scelfo et al. 2009)). Further, abnormal microstructural integrity of cerebellar tracts has been found in other neurodevelopmental disorders with differences in social function; e.g. in people with Asperger syndrome and schizophrenia (Okugawa, Nobuhara et al. 2006; Catani, Jones et al. 2008). Thus, I do not suggest that abnormalities in PFC-cerebellar connections are specific to CD. Rather, they may underlie some aspects of social cognition deficits in a number of neurodevelopmental disorders. Nevertheless, these studies of other disorders only reported abnormality in selective tracts within the cortical-cerebellar-thalamic-cortical network, or of increased FA in some tracts but decreases in others. For example, reduced FA of the superior cerebellar

peduncle was reported in Asperger syndrome, in the absence of deficits in cerebellar input pathways. It was suggested this may contribute to difficulties in social behaviour through deficient cortical input from the cerebellum (Catani, Jones et al. 2008). Further, in people with schizophrenia, increased FA of the superior cerebellar peduncle, which was associated with reduced overall cognitive ability, was found alongside reduced FA of the middle cerebellar peduncle, which contains afferent fibres (Okugawa, Nobuhara et al. 2006). Conversely, I found uniformly increased FA in all major cortical-cerebellar connections in CD compared with controls (i.e. the cerebellar peduncles, corticopontocerebellar tract, corticospinal tract, cerebellar white matter). This suggests that a more generalized cortico-cerebellar 'dysconnectivity' (i.e. 'abnormal functional integration of brain processes' (Klaas, Friston et al. 2009)) may underlie the emotional and behavioural deficits that characterise CD. Support for this suggestion comes from studies that report concurrent abnormality of both prefrontal and cerebellar anatomy (Huebner, Vloet et al. 2008; De Brito, Mechelli et al. 2009; Fahim, He et al. 2011) or function (Rubia, Smith et al. 2009; Passamonti, Fairchild et al. 2010) in young people with CD. Taken together, my work and that of others support the conjecture that boys with CD have abnormal cortico-cerebellar 'connectivity'. It is unknown, however, which comes first (i.e. differences in brain anatomy or function, or behaviour).

As well as increased FA of projection paths, the CD group also showed higher FA in the right anterior thalamic radiation. This limbic tract projects from the

thalamus through the anterior limb of the internal capsule to the frontal and anterior cingulate cortices. Also, fibres in the anterior thalamic radiation project between limbic structures and the frontal cortex (Mori, Wakana et al. 2005; Singh 2006). The authors of a study of high functioning autistic boys reported that significantly reduced FA of the anterior thalamic radiation reflects fronto-thalamic hypofunction (Cheon, Kim et al. 2011). Thus, as a conduit for emotion-related information to/from the frontal lobes, increased FA of the anterior thalamic radiation in my study may reflect aberrantly weighted activation (i.e. hyperfunction or hypofunction) of frontal brain regions by emotional input, such as threat cues. While the current data is not sufficient to verify this, a recent Positron Emission Tomography (PET) study showed that cerebellar damage is associated with abnormal reactivity to emotional stimuli in frontal and limbic regions (Turner, Paradiso et al. 2007). Specifically, fear provoking stimuli produced significantly increased activation of vmPFC, cingulate gyrus and insula, and reduced amygdala/limbic activation. The authors suggested that cerebellar structural deficits prevent the recruitment of structures that typically respond to threat (i.e. the amygdala), and that this results in the recruitment of an alternate circuit (i.e. vmPFC, cingulate gyrus, and insula) (Turner, Paradiso et al. 2007). Children with CD and CU traits show reduced BOLD activation of the amygdala to threat-related stimuli, such as fearful faces (Marsh, Finger et al. 2008; Jones, Laurens et al. 2009). Further, antisocial adults with psychopathy also show reduced amygdala activation to affective stimuli, and this is concurrent with increased activation in the fronto-temporal cortex (Kiehl, Smith et al. 2001). Taken together, my results support the view that abnormalities

within the cerebellum, and specifically white matter connections, may contribute towards the re-organisation of emotion-processing networks in children with CD. Combining DT-MRI with functional imaging techniques could test this hypothesis.

In addition to these regions, the corticospinal tract showed between group differences in FA in my study. It is unclear how the greater microstructural integrity of this tract found in CD may relate to antisocial behaviour. However, one study suggests the involvement of the corticospinal tract in emotion processing, despite it containing predominantly motor axons (Schutter, Hofman et al. 2008). Using transcranial magnetic stimulation, they reported that in healthy controls threat signals, such as fearful facial expressions, increased the motor evoked potential of the corticospinal tract significantly more than positive or neutral faces (Schutter, Hofman et al. 2008). This suggests that the corticospinal tract interacts closely with the limbic system to coordinate the motor response to threat-related stimuli. In this context, increased FA of this tract in CD may indicate abnormality in responding to such cues. This suggestion fits with reports of reduced neural responsivity to fearful faces and affective stimuli in both children with CD/CU traits (Sterzer, Stadler et al. 2005; Marsh, Finger et al. 2008; Jones, Laurens et al. 2009; Passamonti, Fairchild et al. 2010), and adults with ASPD and psychopathy (Kiehl, Smith et al. 2001; Birbaumer, Veit et al. 2005; Deeley, Daly et al. 2006). Future studies could investigate this relationship through correlating emotion processing measures, evoked potentials, and FA of the corticospinal tract.

Together, the tracts I found to be abnormal in CD connect prefrontal-thalamic-cerebellar structures. These regions are also abnormal in adolescents (De Bellis, Narasimhan et al. 2005) and adults with alcohol use disorders (Pfefferbaum, Sullivan et al. 1997; Nicolas, Fernandez-Sola et al. 2000; Sullivan 2003). These disorders, alongside early alcohol use, are predicted by childhood antisocial behaviour (Cadoret, Yates et al. 1995; Lynskey and Fergusson 1995). Moreover, it has been suggested that these disorders arise from a common pathway (Cadoret, Yates et al. 1995). Therefore, disruption to the prefrontal-thalamic-cerebellar white matter pathways in CD may be related to, or even underlie, the structural abnormalities seen in AUDs. Alternatively, the abnormalities I found in CD may be confounded by differences in alcohol use. However, this is unlikely to fully explain the differences I found, as the CD and control samples in my study did not differ in substance and alcohol use. Thus, abnormalities in these tracts in a) young people with alcohol use disorders and b) young people with antisocial behaviour may reflect shared biological determinants affecting white matter microstructure moderating risk of alcohol misuse and antisocial behaviour, respectively.

A final area showing increased FA in CD corresponded with one of the subsections of the superior longitudinal fasciculus (SLF1), which connects the dorsal and medial parietal lobe with the dorsal and medial frontal lobe (Makris, Kennedy et al. 2005). The precise function of this tract is not clear, as *in vivo* investigation of its anatomical characteristics has not long been possible (Jang

and Hong 2011). However, the SLF1 is associated with the updating of verbal, as well as spatial, information; and deficits in verbal intelligence are noted in children with CD (Donnellan, Ge et al. 2000). Thus, increased FA in this tract in CD may interfere with normal information processing and manifest as a verbal cognitive deficit. Investigations using DT-MRI tractography to correlate SFL1 integrity with verbal IQ scores may verify this.

Finally, the results of the behavioural analysis suggest that some of the regions in which greater FA was reported in CD may contribute towards the generation of the emotional and behavioural features of this disorder. While antisocial behaviour measures and FA were not correlated within the CD group, there was a significant positive correlation in the sample as a whole. This suggests that the regions of increased FA in CD are dimensionally associated with antisocial behaviour, but that the small sample size in this study may have prevented the correlations within each group from reaching significance. Support for this is provided by Figure 6.2, which exemplifies the non-skewed distribution of SDQ and APSD scores against FA.

In summary, as outlined above, the between group differences reported in this study were mainly along projection paths. Of all the fibre bundles, the projection tracts are known to show the greatest intensity on DT-MRI images, and thus higher FA, in all brains. This supports what is known about these tracts: that they are dense, myelinated, and compact. Developmental studies have established that the processes underlying these properties (i.e.

myelination, alterations in synaptic density, and increased axon diameter) - alongside FA increases - are an indication of white matter maturation (Hagmann, Sporns et al. 2010). In healthy human populations projection tracts, such as the corticospinal tract, typically become mature by adolescence (Asato, Terwilliger et al. 2010) or the early 20s (Lebel, Walker et al. 2008). Maturation of these tracts corresponds with greater functional connectivity, which in turn reflects increased integration of brain functions connected by the tract (Hagmann, Sporns et al. 2010). While my study found that correlations between FA and age did not significantly differ between groups, there was evidence to suggest that the expected increase in FA that occurs with age in healthy controls is not present in boys with CD. As age-related FA increase in these regions is a hallmark of normal development (Barnea-Goraly, Menon et al. 2005), this finding tentatively suggests that microstructural development may follow an abnormal path in CD - namely that the aforementioned maturational processes may occur at an accelerated rate in the CD group as compared to typically developing boys. Longitudinal studies are needed to explore this hypothesis.

6.6 Limitations

It is important to point out several limitations of this study. Firstly, it should be noted that methodological factors may have contributed to the observed increased FA values I have reported. For example, DT-MRI derived outputs are affected by the issue of 'crossing fibres' (Descoteaux and Deriche 2008). This

is the situation where fibres from tracts following two or more different trajectories inhabit the same voxel. Both fibre bundles will “pull” the diffusion tensor in the direction of principal diffusion along that tract; this artificially distorts the FA in such a way that it may no longer be a meaningful index of white matter integrity. For example, within the right corticospinal tract of Figure 6.1 (vii) there is an area that shows no significant FA difference flanked by two areas that do (corresponding to the corticospinal tract). It is possible either that the area showing no FA difference indeed does not differ between groups or, alternatively, that this is a point at which the bundle is intercepted by fibres passing in a perpendicular direction (i.e. crossing fibres). Therefore, in my study, the tracts showing increased FA in CD may not in fact possess greater microstructural integrity, but rather may have significantly fewer crossing fibres passing across them than control boys. Techniques are now becoming available to minimise this effect, and future studies would benefit from application of these (Dell'Acqua, Rizzo et al. 2007).

In addition, it is possible that increased FA in CD may reflect, rather than acceleration of the progressive process of myelination, a dysfunction in the regressive process of axonal pruning. Axonal pruning removes surplus neuronal processes laid down in earlier developmental stages, in order to refine information processing. However, it is not possible to verify this based on the current data.

6.7 Conclusion

In summary, while the CD group showed increased FA in areas corresponding with the projection tracts, the functional significance of these findings is not clear, due to technical and methodological limitations with current DT-MRI methods. However, despite these limitations my study has identified areas showing significantly different white matter integrity in CD compared to controls. Future studies with larger sample sizes should use tractography within the projection pathways, which could then be correlated with behavioural and clinical measures.

7: Effects of prenatal stress exposure on limbic-prefrontal white matter integrity and behaviour in childhood

7.1 Abstract

Background: The neurobiological basis of antisocial behaviour is poorly understood, but it may involve developmental disruption in limbic-prefrontal white matter circuits. In chapter 5 of the thesis I reported that children with Conduct disorder have significant differences in microstructural integrity of the uncinate fasciculus (UF) compared to their healthy peers. The UF is the major limbic-prefrontal white matter fibre tract that links emotional and social brain regions, and is also abnormal in antisocial adults. The biological determinants of UF maldevelopment are poorly understood. Nevertheless, one potential candidate is maternal antenatal stress, because animal studies suggest that maternal cortisol modulates foetal brain development, and human studies report an association between maternal antenatal stress and conduct problems in their offspring. No study, however, has previously examined white matter integrity in children of mothers in whom measures of both antenatal stress levels and *in utero* cortisol concentration are available. Therefore, I tested the hypothesis that maternal prenatal stress is associated, in their child, with variation in microstructural integrity of the UF and/or behaviour problems.

Methods: From a cohort of the offspring of mothers recruited during pregnancy, 18 healthy children aged between 6 and 9 years old underwent diffusion weighted magnetic resonance imaging (DT-MRI) and behavioural assessment. I explored associations between antenatal stress levels, *in utero* cortisol concentration, and (i) indices of white matter integrity (fractional anisotropy (FA)

and perpendicular diffusivity (D_{perp})) of the UF and a 'control' tract (the Inferior Longitudinal Fasciculus (ILF)); and (ii) child behaviour problems at 6-9 years old.

Results: Maternal antenatal stressful life events were positively correlated, in their child, with right UF FA (r=0.579; p=0.012), and negatively correlated with right UF D_{perp} (r=-0.471; p=0.048). *In utero* cortisol was negatively correlated with FA of the left UF (r=-0.496; p=0.036), and positively correlated with left UF D_{perp} (r=0.515; p=0.029). In addition, there was a trend towards a negative association between *in utero* cortisol and FA in their child's right UF (r=-0.432; p=0.073). There were no associations between; 1) prenatal stress variables and integrity of the ILF control tract or; 2) UF integrity and behaviour problems at age 6-9 in this non CD population of healthy children. Sociodemographic and obstetric variables were not associated with integrity of either tract.

Conclusion: These findings tentatively support the hypothesis that in humans maternal prenatal stress modulates brain development of healthy children; and this includes white matter tracts that form part of the brain networks underlying social behaviour.

7.2 Introduction

The two previous chapters of this thesis presented data suggesting that adolescents with CD have abnormally increased white matter 'connectivity' as compared to typically developing youngsters, and that this may underlie their emotional processing and social behaviour deficits. Specifically, I reported that adolescents with CD have significant differences in the microstructural integrity of the major limbic-prefrontal pathway, the uncinate fasciculus (UF), which connects emotional and social brain regions, and is also abnormal (reduced) in antisocial adults (reported in Chapter 5; Craig, Catani et al. 2009; Motzkin, Newman et al. 2011; Sundram, Deeley et al. 2011). Increased FA was also found within cerebellar connections (reported in Chapter 6). It has been proposed by some that greater FA reflects precocious brain maturation, which may lead adolescents to engaging in activities more suitable for adults (Berkowitz 1993; Berns, Moore et al. 2009). However, it is unknown how the abnormalities I detected may have arisen.

It is likely that the explanation for these differences is complex, and includes a large variety of genetic and environmental factors. Nevertheless, one prominent candidate may be maternal stress - because there is increasing evidence that a mother's emotional state during pregnancy can affect their child's behavioural development. For example, many studies have reported that, after controlling for postnatal factors, antenatal maternal stress and anxiety are associated with increased rates of conduct problems and temperament

difficulties in offspring (Huizink, de Medina et al. 2002; O'Connor, Heron et al. 2002b; O'Connor, Heron et al. 2003; Gutteling, de Weerth et al. 2005b; Barker and Maughan 2009; Rice, Harold et al. 2010). Further, maternal antenatal depression has been linked to later antisocial behaviour in their teenage children (Hay, Pawlby et al. 2010). Together these studies suggest an association between high levels of antenatal maternal stress and/or depression and long term behavioural effects in their offspring.

In addition to an association with externalising behaviour, a mother's mood has also been reported to affect her child's emotional reactivity. For example, the number of stressful life events a mother experienced in pregnancy is positively correlated with infant fearfulness at ~18 months (Bergman, Sarkar et al. 2007). Further, higher emotional reactivity in children is associated with impulsivity, and conduct problems (Frick, Cornell et al. 2003b; Loney, Frick et al. 2003) – which in turn are associated with their developing CD (Loeber, Green et al. 1995). Finally, antenatal maternal depression is also positively associated with infantile negative reactivity (Davis, Glynn et al. 2007), which may also be a risk factor for behaviour problems (Bates, Freeland et al. 1979; Snyder, Reid et al. 2003). Together these studies highlight a potential link between maternal stress/mood and their infant's subsequent development of emotionality, impulsivity, and childhood conduct problems.

It is unclear what drives the association between maternal prenatal factors and their child's behaviour. Nevertheless it has been suggested that the endocrine environment may play a key role – by modulating brain development. For

instance, neuroanatomical abnormalities in temporo-limbic and prefrontal regions have been reported in offspring exposed antenatally to maternal stress and/or glucocorticoids (Uno, Eisele et al. 1994; Salm, Pavelko et al. 2004; Kraszpulski, Dickerson et al. 2006; Buss, Davis et al. 2010; Tamura, Sajo et al. 2011). Further, as discussed in Chapter 2, deficits in the structure and function of limbic and prefrontal grey matter are associated with childhood behaviour problems (Kruesi, Casanova et al. 2004; Sterzer, Stadler et al. 2005; Sterzer, Stadler et al. 2007; Herpertz, Huebner et al. 2008; Huebner, Vloet et al. 2008; Marsh, Finger et al. 2008a; De Brito, Mechelli et al. 2009; Jones, Laurens et al. 2009; Rubia, Smith et al. 2009; Passamonti, Fairchild et al. 2010; Fahim, He et al. 2011; Fairchild, Passamonti et al. 2011). Therefore, it is possible that antenatal factors contribute towards neurodevelopmental abnormalities affecting the limbic and prefrontal regions, potentially leading to conduct problems in childhood.

This suggestion is supported by reports that limbic brain abnormalities arise from increased prenatal stress and/or glucocorticoid exposure in rats and monkeys. For example, antenatal maternal stress in rats results in higher cell counts and altered development of amygdaloid nuclei (Salm, Pavelko et al. 2004; Kraszpulski, Dickerson et al. 2006) in their offspring – and these differences are correlated with fearfulness (Kraszpulski, Dickerson et al. 2006). Also, dendritic complexity and spine density of hippocampal granule cells is reduced in prenatally stressed rats (Tamura, Sajo et al. 2011), and reduced hippocampal volume has been reported in the offspring of prenatally stressed monkeys (Uno, Eisele et al. 1994). Finally, a single human study reported that

children of prenatally anxious mothers have reduced grey matter density in brain regions including the temporal lobe, PFC, and cerebellum (Buss, Davis et al. 2010). In summary, these studies suggest that prenatal stress modulates the development of limbic grey matter regions, such as the hippocampus and amygdala. However, these brain regions do not function in isolation – rather they are linked by white matter tracts to form part of the 'limbic system'.

Research shows that prenatal stress also affects the development of white matter. For example, in rats restraint stress administered during gestation results in early hypermyelination in the brains of their pups (Wiggins and Gottesfeld 1986). Further, in sheep prenatal administration of corticosteroids results in reduced myelination, axon diameter, and myelin thickness in corpus callosum (Huang, Harper et al. 2001) and slower rates of axonal myelination in foetal optic nerve (Dunlop, Archer et al. 1997).

In summary, prior studies suggest that prenatal stress/elevated maternal glucocorticoid exposure modulates the development of foetal grey matter regions and white matter connections in the brain. The mechanism by which this occurs remains unclear, but may include increased transfer of maternal glucocorticoids (i.e. cortisol/corticosterone) into the foetal environment. Also, antenatal maternal stress/intrauterine glucocorticoid exposure is associated with an increased risk of conduct problems in offspring, and with the abnormal development of the same brain regions found to be abnormal in children with CD (i.e. PFC and temporal-limbic cortices). Animal studies indicate that white matter development may be vulnerable to the effects of prenatal adversity

(Wiggins and Gottesfeld 1986; Dunlop, Archer et al. 1997; Huang, Harper et al. 2001). However, to date no human study has examined white matter integrity in children with known levels of prenatal stress and/or cortisol exposure. Therefore, I carried out a preliminary investigation to test the hypothesis that exposure to antenatal maternal stress and/or elevated *in utero* cortisol is associated within children with variation in microstructural integrity of limbic white matter tracts I have previously reported as developmentally abnormal in CD.

The UF white matter tract connects limbic and prefrontal brain regions; and in Chapter 5 I reported that antisocial children have significant differences in the microstructural integrity of the UF as compared to healthy children. Hence, in this study, I used DTI tractography (see Chapter 4 for a full description of DT-MRI) to assess white matter integrity of the UF in typically developing 6 to 9 year old children who had previously been exposed to varying levels of maternal antenatal stress, and in whom intrauterine cortisol concentration was measured during pregnancy. I examined the association between limbic-prefrontal 'connectivity' and (i) measures of prenatal stress (antenatal stressful life events, and/or *in utero* cortisol concentration); (ii) antisocial behaviour measures at age 6-9 years (conduct problems, callous-unemotional traits, narcissism, and impulsivity); and (iii) measures of temperament (fear reactivity) that had been obtained at ~18 months. Associations between DT-MRI indices and postnatal maternal mood (depression), and several socio-demographic and obstetric variables were also examined.

7.3 Materials and methods

7.3.1 Participants

The participants included eighteen children between 6 and 9 years old from an existing cohort who were originally recruited by Imperial College London (see Materials and Methods Chapter 4 for full details; Table 7.1). The participants recruited into the current study did not differ significantly from the pool from which they were drawn on any prenatal or obstetric measures.

Table 7.1: Group characteristics of children

N=18	**Mean (SD)**
Age in years	8 (1)
Mean FSIQ	121 (11)
Conduct problems (SDQ)	1 (1)
Hyperactivity (SDQ)	3(2)
Emotional Problems (SDQ)	2(3)
Peer Problems (SDQ)	1(2)
Prosocial Behaviour (SDQ)	9(2)
Total problems (SDQ)	7(5)
Callous-unemotional traits (APSD)	3(1)
Narcissism traits (APSD)	2(2)
Impulsivity (APSD)	4(1)
Total score (APSD)	8(5)
Fear reactivity score (LabTAB)	6(4)
Prenatal stress measures (n=18)	**Mean (SD)**
Number of stressful life events (SLE)	1(1)
In utero cortisol concentration (nM/L Log)	3(0)
Postnatal maternal measures	**Mean (SD)**
Edinburgh Postnatal Depression Scale score	9 (1)
Ethnicity (n=18)	**%**
White	78
Black/African-Caribbean	6
Other	17

Annual income (n=15)	**%**
Below £18,000	0
£18,000-£25,000	0
£25,000-£43,000	40
Above £43,000	40
Prefer not to say	20
Maternal highest level of education (n=18)	**%**
No qualifications	6
GCSEs	6
A levels/third level	89
Obstetric variables (n=18)	**Mean (SD)**
Maternal age in years	36 (4)
Birth weight (grams)	3410 (376)
Smoking in pregnancy (cigarettes per day)	Number of mothers
0	16
1-2	2
Alcohol intake in pregnancy (units per week)	Number of mothers
0	12
1	6
Sex of child (n=18)	**N**
Females	10
Males	8

SDQ – Strengths and Difficulties Questionnaire; APSD – Antisocial Process Screening Device; SD – standard deviation; LabTAB – Laboratory Temperament Assessment Battery; nM/L – nanomoles per litre; Log – log-transformed

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7.3.2 Measures

For full details of measures please see Methods Chapter 4.

7.3.2.1 Questionnaires

7.3.2.1.1 Strengths and Difficulties Questionnaire (SDQ) – parent report

The SDQ is a 25-item questionnaire consisting of subscales used to assess: conduct problems, hyperactivity, emotional problems, peer problems, and prosocial behaviour. For full description of this questionnaire please refer to Methods Chapter 4.

7.3.2.1.2 Antisocial Process Screening Device (APSD) – parent report

The APSD is a 20 item questionnaire that assesses antisocial traits, namely: callous-unemotional traits, narcissism, and impulsivity. For a full description of this questionnaire please refer to Methods Chapter 4.

7.3.3 DT-MRI data

DT-MRI data were acquired and preprocessed as described in Methods Chapter 4. I also performed tractography using TrackVis software using the same methods as were applied in Chapter 5 of this thesis. In this study the tract of interest was the UF, and the inferior longitudinal fasciculus (ILF) was selected as a control tract as this tract shares the UF's limbic connection, while not connecting to the frontal lobe.

7.3.4 Statistical analysis

7.3.4.1 Prenatal stress indices and DT-MRI measures

Due to the small sample size, and as several of the covariates did not meet the assumptions for a parametric test, it was not possible to use a regression model to analyse these data. Thus, this constitutes a pilot investigation using multiple non-parametric correlations. Spearman's rho bivariate analyses were carried out to examine correlations between two measures of white matter microstructure of the UF and control tract (namely, fractional anisotropy (FA), and perpendicular diffusivity (D_{perp})), and (1) antenatal stressful life events (SLE); and (2) *in utero* cortisol concentration (CORT). Postnatal depression (assessed using the Edinburgh Postnatal Depression Scale (EPDS; Cox, Holden et al. 1987) at approximately 18 months after birth) was also correlated with UF DT-MRI indices to determine whether there was any confounding

influence of postnatal factors on UF integrity. In addition, correlations between DT-MRI indices and other possible confounders (child age, maternal age, birth weight corrected for gestational age, smoking in pregnancy, and SES) were also examined to ensure these were not associated with the UF DT-MRI outcome measures.

7.3.4.2 Childhood behaviour and DT-MRI measures

Where significant associations were identified between DT-MRI indices and SLE, cortisol, or postnatal depression, further correlation analysis investigated whether these were associated with the following childhood behavioural measures: (1) conduct problems; hyperactivity/Inattentiveness; and total problems SDQ subscales; and (2) callous-unemotional traits; impulsivity; and total score on the APSD questionnaire.

7.3.4.3 Infant fear reactivity and DT-MRI measures

Further correlation analysis examined whether UF DT-MRI indices were associated with fear reactivity that had been measured during infancy, at ~18 months.

7.3.4.4 Infant fear reactivity and childhood behaviour

Spearman's correlation analysis was also used to investigate whether infant fear reactivity assessed at ~18 months was associated with current antisocial behaviour, as assessed using the SDQ and APSD.

7.4 Results

7.4.1 Prenatal stress indices and DT-MRI measures

Maternal SLE were correlated positively with their offspring's right UF FA (r=0.58; p=0.012), and negatively with right UF D_{perp} (r=-0.47; p=0.048). In contrast, *in utero* cortisol concentration was inversely correlated with FA of the left UF (r=-0.50; p=0.036); and positively correlated with D_{perp} of this tract (r=0.52; p=0.029).

However, stressful life events and *in utero* cortisol concentration were not correlated (r=-0.17 p=0.493). No sociodemographic or obstetric factors correlated with UF DT-MRI outcome variables. No associations were found between maternal antenatal measures and the ILF control tract. See Table 7.2; Figure 7.1.

7.4.2 Postnatal depression and UF integrity

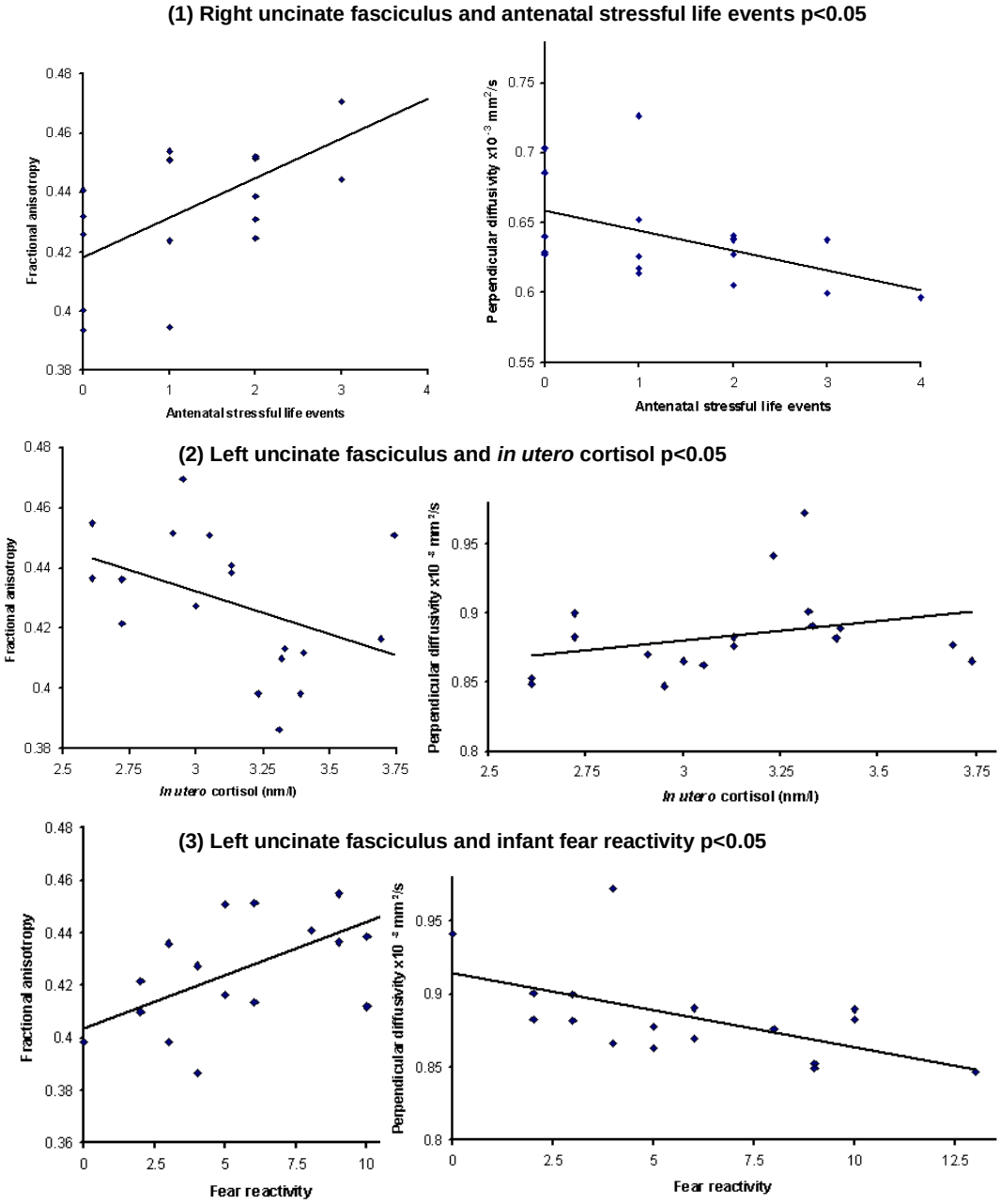
There were no significant correlations between UF DT-MRI indices and maternal postnatal depression.

Table 7.2: Correlations between *in utero* cortisol concentration, antenatal stress, postnatal depression, infant fear reactivity, and DT-MRI indices

Tract	Hemisphere	Value	*In utero* cortisol (nm/l)		Antenatal stressful life events		Maternal postnatal depression		Infant fear reactivity ~18 months	
			r	p	r	p	R	p	r	p
Uncinate fasciculus	FA	Left	-0.50	0.036*	0.29	0.249	0.09	0.723	0.61	0.010*
		Right	-0.43	0.073	0.58	0.012*	0.03	0.893	0.15	0.578
	D_{perp}	Left	0.52	0.029*	-0.34	0.166	-0.14	0.592	-0.58	0.014*
		Right	0.37	0.135	-0.47	0.048*	-0.01	0.958	-0.29	0.256
Inferior longitudinal fasciculus	FA	Left	0.17	0.510	0.07	0.775	-0.24	0.331	-0.04	0.866
		Right	-0.14	0.576	0.18	0.466	-0.11	0.656	0.12	0.645
	D_{perp}	Left	0.06	0.823	-0.19	0.446	0.08	0.766	-0.09	0.728
		Right	0.21	0.409	-0.25	0.322	0.11	0.671	-0.17	0.511

*FA – fractional anisotropy; D_{perp} – perpendicular diffusivity; r – correlation coefficient; p – significance level; * p<0.05 (2-tailed)*

Figure 7.1: Significant correlations between uncinate fasciculus fractional anisotropy and perpendicular diffusivity, and in utero cortisol, antenatal stressful life events and infant fear reactivity



All significant correlations shown within the uncinate fasciculus (1) antenatal stressful life events and right hemisphere FA (*left figure*); D_{perp} (*right figure)*; (2) *in utero* cortisol and left hemisphere FA (*left figure)*; D_{perp} (*right figure)*; and (3) infant fear reactivity and left hemisphere FA (*left figure*); D_{perp} (*right figure*).

7.4.3 Childhood behaviour and DT-MRI measures

No significant correlations were found between UF DT-MRI and childhood antisocial behaviour measures (Table 7.3).

Table 7.3: Correlations of tracts with antisocial behaviour measures

Tract	Hemisphere	Value	Conduct problems SDQ		Hyperactive/ inattentive SDQ		Total problems SDQ		Callous unemotional traits APSD		Impulsivity APSD		Total score APSD	
			r	p	r	p	r	P	r	p	r	p	r	p
Uncinate fasciculus	FA	Left	-0.30	0.222	-0.15	0.558	-0.30	0.231	0.23	0.370	0.28	0.255	0.29	0.236
		Right	-0.24	0.332	-0.22	0.381	-0.24	0.348	0.06	0.806	0.26	0.305	0.03	0.916
	D_{perp}	Left	0.33	0.179	0.06	0.825	0.17	0.495	-0.00	0.993	0.28	0.255	-0.16	0.519
		Right	0.27	0.074	0.27	0.274	0.25	0.310	-0.05	0.852	-0.24	0.329	-0.12	0.961

FA – Fractional anisotropy; D_{perp} – Perpendicular diffusivity; SDQ – Strengths and Difficulties Questionnaire; APSD – Antisocial Process Screening Device; r – correlation coefficient; p – two-tailed significance level

7.4.4 Infant fear reactivity and DT-MRI measures

Infant fear reactivity assessed at ~18 months was significantly correlated with left UF values (Table 7.2; Figure 7.1); correlating positively with FA (r=0.61; p=0.010), and negatively with D_{perp} (r=-0.58; p=0.014).

7.4.5 Infant fear reactivity and childhood behaviour

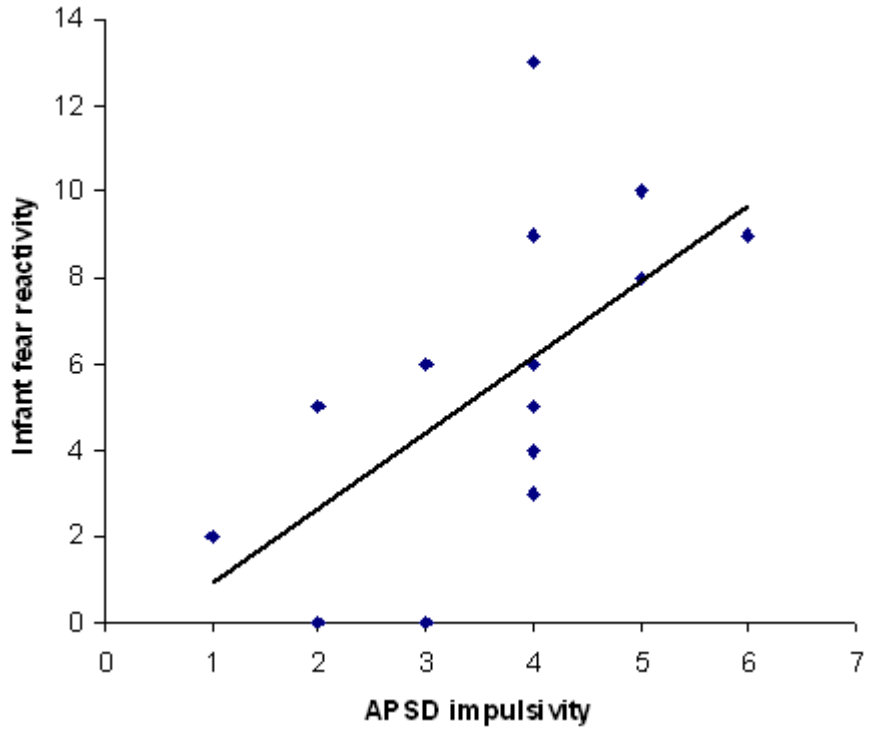
There was a significant positive correlation between fear reactivity in infancy, and impulsivity at age 6-9 years old (r=0.71; p=0.002; Table 7.4; Figure 7.2).

Table 7.4: Correlations between infant fear reactivity and childhood antisocial behaviour measures

Instrument	Subscale	Infant fear reactivity ~18 months	
		R	p
SDQ	Conduct problems	-0.18	0.48
	Hyperactivity/inattentive	0.20	0.45
	Total problems	-0.09	0.74
APSD	Callous-unemotional traits	0.08	0.76
	Impulsivity	0.71	0.00*
	Narcissism	0.03	0.92
	Total problems	0.41	0.10

*SDQ – Strengths and Difficulties Questionnaire; APSD – Antisocial Process Screening Device; r – correlation coefficient; p – significance level; *p<0.01 (2-tailed).*

Figure 7.2: Significant correlation between infant fear reactivity and childhood impulsivity (p<0.01)



APSD – Antisocial Process Screening Device

7.4.6 *Post hoc* analysis of UF DT-MRI indices and age

Age and UF DT-MRI indices were not significantly correlated, but did mirror the relationship seen in typical (i.e. healthy) aging (i.e. FA increases and D_{perp} decreases with age) reported by others (Lebel, Walker et al. 2008).

7.5 Discussion

7.5.1 Stressful life events and the UF

In this study I found significant correlations between self report measures of maternal prenatal stress exposure and two different indices of microstructural integrity of the right UF (the white matter tract connecting the PFC with the anterior temporal lobe and amygdala) in their children aged between 6 and 9 years old. There were no associations between UF integrity and sociodemographic, obstetric, or postnatal measures. This suggests that the correlations between maternal prenatal stress and the microstructure of this white matter tract in their child were independent of sociodemographic and obstetric risk factors and of maternal postnatal mood. Further, the associations were tract specific (i.e. they were not found in the control tract).

Antenatal stressful life events showed a significant positive correlation with FA, but a negative correlation with D_{perp}, of the right UF. FA and D_{perp} are indices of tract integrity, which are, in turn, influenced by degree of myelination, and inter- and intra-axonal factors (e.g. axon diameter, and fibre number) (Beaulieu 2009; Paus 2010). FA assesses longitudinal diffusivity of water molecules along the length of the axon, whereas D_{perp} reflects diffusion occurring radially, across the axon. While both FA and D_{perp} reflect all of the aforementioned axonal properties, D_{perp} has been found to be particularly indicative of myelination, being inversely related to myelin content (Song, Yoshino et al. 2005; Avram,

Guidon et al. 2010). FA normally increases with age, reaching its maximal values in early adulthood (Lebel, Walker et al. 2008), whereas D_{perp} typically decreases with age (Lebel and Beaulieu 2011). Thus, the reported positive correlation between prenatal stress and the right UF FA, and reduced $D_{perp,}$ tentatively suggests that exposure to greater numbers of antenatal stressors may be associated with increased, or accelerated, aging of the UF. However, *post hoc* examination of the relationship between DT-MRI measures and age did not find correlations to be statistically significant.

Early maturation (or increased aging) of the UF may be the result of increased myelination, a key determinant of FA and D_{perp} values, along with greater fibre number and/or diameter (Lebel, Walker et al. 2008; Beaulieu 2009). Myelination begins during foetal life and continues until early adulthood (Marsh, Gerber et al. 2008), and this process corresponds to the increased FA and decreased D_{perp} seen with typical aging (Lebel, Walker et al. 2008). Reduced D_{perp} is understood to particularly reflect increasing axonal myelin content, as myelin prevents the radial diffusion of axonal water molecules while permitting, and increasing, longitudinal diffusivity (i.e. reflected by greater FA) (Avram, Guidon et al. 2010). While it is not clear whether maternal antenatal stress increases myelination in humans, a single animal study suggests that this may occur. This reported that, in rats, maternal restraint stress applied during the late stages of gestation is associated with hypermyelination in the brain of their offspring (Wiggins and Gottesfeld 1986). If hypermyelination also occurs in children as a result of maternal exposure to greater numbers of antenatal stressful life events this could account for the greater FA, and reduced D_{perp}, I

detected in the UF. However, I was unable to address this issue *directly* as I did not measure myelination of the UF *per se* (e.g. using new myelin mapping techniques) (Deoni, Mercure et al. 2011). Future studies could, however, investigate whether maternal stressful events are associated with differences in myelination of their infant's brain. Finally, it should be considered that while increased FA and reduced D_{perp} may reflect abnormally increased tract maturation, they may also point to other factors. For example, as white matter pathways develop they become increasingly complex and show increased branching; and it has been proposed that abnormality of branching processes may contribute towards increased FA (Allin 2010). Future studies are needed to explore this further (e.g. using animal models).

7.5.2 *In utero* cortisol and the UF

The second association I found in this study was of a significant inverse relationship between *in utero* cortisol concentration and microstructural integrity of the left UF. It is important to note that this relationship was in the opposite direction to that observed between UF FA and antenatal stressful life events. It may be thought likely that if the effect of stressors on UF is mediated by cortisol, then the effect would share the same directionality as stressful life events; however, I found that stressful life events and cortisol were not correlated to each other. There are at least two ways to interpret this finding.

Firstly, it is possible that high levels of stress at around 17 weeks of gestation (i.e. the stage of gestation at which the sample was obtained) do in fact correspond to low levels of *in utero* cortisol. This is because the association between levels of maternal antenatal stress exposure, human maternal circulatory cortisol, and levels found *in utero*, is neither straightforward nor linear (Seckl 2001; Obel, Hedegaard et al. 2005; Diego, Jones et al. 2006; Sarkar, Bergman et al. 2006; Ventura, Gomes et al. 2012). For example, studies that have examined the relationship between maternal anxiety/stress and maternal cortisol secretion report only weak to modest correlations (Diego, Jones et al. 2006; Sarkar, Bergman et al. 2006; Ventura, Gomes et al. 2012) that are, further, highly variable between women (Seckl 2001). Also, there are differential associations between maternal mood and circulating maternal cortisol depending on gestational stage, with late pregnancy stress/anxiety being associated with higher cortisol levels as compared to early pregnancy (Obel, Hedegaard et al. 2005). One reason for this is that the majority of cortisol (80-90%; Seckl 2004) is metabolized before crossing into the womb by the placental enzyme 11ß-HSD-2 (11 Beta-hydroxysteroid dehydrogenase-Type 2; Glover 1997). In early pregnancy 11ß HSD2 ensures that *in utero* levels of cortisol are far lower than maternal circulatory levels (Fowden and Forhead 2004). Thus, this work provides one potential explanation for the divergent associations of UF FA to stressful life events and *in utero* cortisol.

A second interpretation of low *in utero* cortisol corresponding to higher UF microstructural integrity centres on the nature of the stressors observed in this study. The stressful life events questionnaire assessed chronic stressors

occurring during pregnancy. Individuals' cortisol response to chronic stress is understood to be highly variable, and is dependent on a number of variables, including personality factors and nature and timing of stress (see Miller, Chen et al. 2007). In contrast, the measure of *in utero* cortisol can be viewed as a measure of acute stress response at a single time point: namely to an imminent amniocentesis. Pregnant women experience high levels of anxiety and stress at the time immediately preceding amniocentesis (Leithner, Maar et al. 2004; Brajenovic-Milic, Martinac Dorcic et al. 2010). A lower cortisol concentration associated with this acute stressor may therefore reflect a 'blunted' maternal stress response. There is growing evidence that low cortisol reactivity may be present where individuals have been exposed to chronic stress (for a review please see Heim, Ehlert et al. 2000; and Gunnar and Vazquez 2001). In other words, mothers who have experienced ongoing stress (e.g. stressful life events) may subsequently show a blunted cortisol response to acute stress of the amniocentesis. Therefore, it is possible that lower *in utero* cortisol may reflect a dampened HPA-axis response due to previous/ongoing chronic stress. Against this suggestion, however, is that I did not find any relationship (positive or negative) between maternal stressful life events and cortisol (although it is not clear if this is due to the relatively small sample size). Future investigations with larger samples could verify whether this is the case.

Another interpretation of the association between low levels of *in utero* cortisol and greater UF maturity may lie with the relationship between cortisol and other HPA-axis hormones. Maternal cortisol secretion is stimulated by both maternal and placental corticosteroid releasing hormone (CRH; pCRH). There is now

evidence that exposure to pCRH, as well as cortisol, is associated with neurodevelopmental changes. For example, low levels of pCRH (which is stimulated by foetal cortisol) in early pregnancy is associated with precocious foetal maturation, as indexed by foetal responses that represent nervous system development (Class, Buss et al. 2008). As secretion of cortisol and pCRH is positively correlated in the second trimester of pregnancy, it is likely that low pCRH concentration corresponded with low levels of cortisol in the Class et al cohort. Accelerated foetal developmental may include greater white matter integrity, which may be reflected by greater FA and reduced D_{perp}. Thus, it is possible that my finding of increased UF FA in relation to lower *in utero* cortisol may be related to precocious development occurring as a result of low pCRH/cortisol concentration. However, without having measured pCRH it is not possible to verify this explanation.

A further consideration in interpreting the increased FA of the UF is the possibility that factors beyond those assessed in this study may also be contributing to white matter development and behaviour. One such factor is serotonin (5), a neurotransmitter that is also involved in the stress response, the development of neural circuits that underlie social cognition (Dinan 1994; Strickland, Deakin et al. 2002; Bonnin, Goeden et al. 2011), and aggression (Virkkunen, Nuutila et al. 1987; Higley, Mehlman et al. 1992; Virkkunen, Rawlings et al. 1994). For example, stress exposure, and consequent hypersecretion of cortisol, is associated with disruption to the 5HT system (Dinan 1994; Strickland, Deakin et al. 2002). Further, a recent animal study reported that the development of foetal forebrain neural circuits may be

modulated by exposure to placenta-derived 5HT (Bonnin, Goeden et al. 2011), potentially affecting the development of systems that underlie mood and emotion (Bonnin and Levitt 2012). Finally, 5HT hypofunction has been associated with aggression in humans and animals (Virkkunen, Nuutila et al. 1987; Higley, Mehlman et al. 1992; Virkkunen, Rawlings et al. 1994). Taken together there is evidence that maternal derived 5HT may modulate white matter development as a function of stress exposure, which may in turn lead to aggressive behaviour in offspring. Future studies are needed to examine this relationship further.

7.5.3 UF and child behaviour

Increased UF FA was not found to be associated with any of the behavioural measures of CD and/or CU traits in childhood. One possibility is that we were able to detect subtle variations in development that are not associated with gross behavioural differences in healthy individuals. Alternatively, low statistical power due to small subject numbers, or limited behavioural measures (e.g. relatively blunt measures with a 'floor effect'), may be the cause of this finding, and future studies should confirm this with a larger sample.

7.5.4 UF and infant behaviour

While there was no association between UF integrity and child behaviour measures at age 6-9 years, there was a significant positive correlation between

left UF FA as assessed at this age and fear reactivity that had been assessed earlier during infancy. Conversely, D_{perp} of this tract correlated inversely with fearfulness – as D_{perp} is typically reduced alongside increased FA. These results suggest there is a positive relationship between limbic-prefrontal 'connectivity' and infant fear reactivity. It is possible that this association may be influenced by stressful life events, as these have been found to be related to both UF FA and to infant fearfulness. First, my study found that stressful life events were positively correlated with left UF FA. Second, a previous study using data from this same cohort had reported a significant positive correlation between antenatal stressful life events and increased infant fearfulness (Bergman, Sarkar et al. 2007). Taken together, there is evidence to suggest that the association between prenatal stress and infant fearfulness may be mediated by the integrity of limbic-prefrontal white matter. However, it is important to note there are three difficulties with this proposal. First, this is an indirect link (i.e. I did not find a direct association between antenatal stressful life events and fearfulness). Second, the association between UF integrity and stressful events was in the opposite hemisphere to that found between UF integrity and infant fearfulness (i.e. right hemisphere rather than left hemisphere, respectively). There was no *a priori* reason to anticipate this hemispheric difference, and it is not clear what the significance of it may be. Last, left UF FA was inversely correlated with *in utero* cortisol. This suggests that low cortisol increases left UF FA, which is positively associated with high fearfulness in infancy (see Figure 7.3). In conclusion, my study provides preliminary evidence that increased infant fear reactivity in babies may be

related to antenatal stressful events, and may possibly also arise through developmental differences in UF.

Figure 7.3: Diagram to show the inter-relationships between prenatal stress, UF integrity and infant fear reactivity

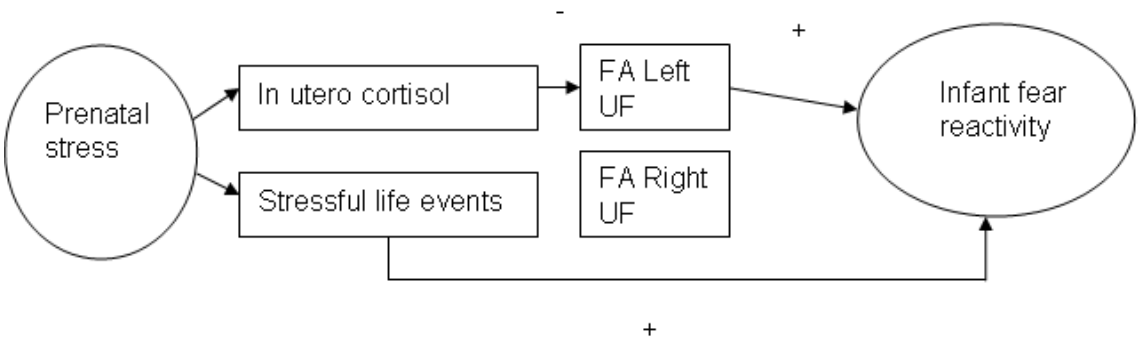


Diagram showing inter-relationships between prenatal stress measures, fractional anisotropy (FA) of the uncinate fasciculus (UF), and infant fear reactivity.

Further support for a neuroanatomical link between prenatal stress and infant fear reactivity comes from animal studies reporting that increased fearfulness in prenatally stressed rats was associated with abnormal amygdala grey matter structure (Kraszpulski, Dickerson et al. 2006). Further, abnormal development of, and higher cell counts within, the amygdala are observed in prenatally stressed offspring (Salm, Pavelko et al. 2004; Kraszpulski, Dickerson et al. 2006). As the amygdala activates robustly to threat, the deficits observed from prenatal stress (i.e. increased fearfulness and amygdala abnormality) indicate potentially greater amygdala involvement in response to threat in these subjects. My study did not find an association between increased UF FA and

behaviour problem scores, but it did find an association with fear reactivity. The behavioural effect of increased perception of, or reactivity to, threat has been reported in antisocial children. Specifically, boys with CD show greater negative attribution towards emotional stimuli than healthy boys (Dadds, Perry et al. 2006), which may reflect greater amygdala responsivity towards fear/threat in CD. Further, reactive aggression has long been understood to be a response to threat (Berkowitz 1993), which triggers fear or anger (Lazarus 1991; Ohman 2005). The increased integrity of the UF may modify the functional efficiency of white matter tracts along which PFC activation suppresses the amygdala, as it ordinarily does (Urry, Van Reekum et al. 2006). For example, electrophysiology studies report that FA correlates positively with neural activity (Begre, Kleinlogel et al. 2008; Westlye, Walhovd et al. 2009), thus increased FA of the UF may result in significantly increased transmission of emotional input to the PFC from limbic structures, and this may exceed the ability of the PFC to adequately suppress this. This could underlie the increased amygdala activation and fearfulness observed in prenatally stressed animals. Further support for the idea that PFC-amygdala coupling is important in CD comes from the suggestion that emotion processing deficits can arise from children's' attempts to reduce amygdala activity (i.e. to perceived, or actual, threat) through the adoption of avoidant strategies; and that these deficits may then lead to conduct problems (Hill, Murray et al. 2008). In summary, despite the associations reported by others between fearfulness, threat response, and CD (Dadds, Perry et al. 2006), my results did not support a direct relationship between prenatal stress measures and antisocial tendencies in children at age 6-9. However, the children in this study were typically developing youngsters, and so scores on

measures of antisocial traits were extremely low and showed limited variance. It is possible, nevertheless, that a small subgroup of the cohort may exhibit antisocial traits at later developmental stages; however, only the longitudinal assessment of behaviour of these children can elucidate whether increased UF FA and increased fearfulness are associated with the development of conduct problems and/or psychopathic traits.

7.5.5 Infant fear reactivity and impulsivity at age 6-9

Although UF DT-MRI indices were not directly associated with child behaviour (see Section 3 above), there was some tentative evidence for an indirect relationship between UF integrity and impulsivity at age 6-9 *via* behaviour measured in infancy. For example, fearfulness at ~18 months (which was positively correlated with left UF FA, see above) was positively associated with impulsivity in children, as rated by the APSD questionnaire. Impulsivity is a known risk factor for the development of CD (Loeber, Green et al. 1995), and is one of the three dimensions used to measure psychopathic traits in children and adolescents (Frick and Hare 2001; Forth, Kosson et al. 2003). Thus, increased scores on this behavioural dimension may indicate that those children with increased fear reactivity may show a propensity towards conduct problems and have greater levels of this developmental psychopathy trait. Tentative support for this suggestion comes from adult neuroimaging studies that reported abnormal microstructural integrity of the UF in psychopathic adults (Craig, Catani et al. 2009; Motzkin, Newman et al. 2011; Sundram, Deeley et al. 2011).

However, it is not possible to extrapolate results from this healthy child sample to antisocial adults. Thus, large scale longitudinal studies are needed to clarify whether increased FA of this tract in childhood underpins the expression of psychopathic traits in later life.

Last, support for an association between increased FA of frontal white matter tracts and impulsivity is provided by a recent study of adolescents; FA was positively correlated with a measure of impulsive and risk-taking behaviour (Berns, Moore et al. 2009). Greater white matter integrity was taken to indicate precocious brain maturation, which could lead adolescents to engaging in activities more suitable for adults (Berns, Moore et al. 2009). Thus, taken together there appears to be an association in youngsters between increased FA of frontal white matter tracts and greater impulsivity. However, as noted above, my preliminary results suggest that the association between increased UF FA and impulsivity may be indirect (via fearfulness in infancy).

7.6 Limitations

It is important to outline a number of limitations with this study. Firstly, the number of children recruited into the study was small, and a greater sample would have increased statistical power. For example, while it would have been desirable to model the relative contribution of each of the covariates and confounding variables using a regression analysis, this was not feasible with the current sample size (Pallant 2007). However, given the difficulties with scanning children from this cohort (e.g. many were below the age of 6 and a half at the time of scanning, most were available only during school holidays so were competing for a limited number of scanning sessions, and several datasets were excluded as they contained excess motion artefact), this was the most possible in the available time.

A second caveat is that correlation analysis with the antenatal stressful life events questionnaire data may have suffered from floor effects. The range of values within my sample was very narrow, and this may have contributed to Type 1 error. As this study constitutes preliminary analysis from a larger dataset (continuing to be collected) this issue should not recur in the eventual study findings.

Another issue that should be considered in interpreting these findings is the demography of the sample studied. For example, mothers were older than the national average (Office for National Statistics, 2010), were mostly white, and

were well-educated. For this reason, the ability to generalise these findings to other samples may be limited.

Finally, it is important to note that while this study has identified correlations (i.e. associations) between brain structure and prenatal events it is not possible to clearly ascribe *causation* or rule out the influence of other factors on these neurodevelopmental outcomes. For example, data were not available that pertained to childhood trauma, negative family experiences, illnesses, and other events/neurochemical mechanisms that may adversely influence brain development throughout infancy and childhood. For this reason, drawing clear conclusions from the findings of this study is not possible. However, this is the first study in which the relationship between prenatal stress, *in utero* cortisol, and white matter has been investigated; for this reason it is an important body of preliminary research within the field of perinatal psychiatry.

7.7 Conclusion

In summary, this study found significant relationships between antenatal stressful life events and *in utero* cortisol concentration and limbic-prefrontal white matter integrity in young children. While it is not clear how reduced cortisol exposure could give rise to the increased white matter properties reflected by increased FA and reduced D_{perp}, there appears to be a relationship between these measures and increased fearfulness in infancy. Furthermore, I found that infant fear reactivity was positively correlated with impulsivity in childhood. Together with the reported increase in FA, these results suggest that increased UF integrity (resulting from increased stressful life events/reduced cortisol) may be related to a fearful temperament in infancy, that then manifests as childhood impulsivity, which is a known risk factor for CD (Loeber, Green et al. 1995). Thus, increased UF FA may indicate a greater susceptibility to conduct problems in childhood. While this study constitutes a preliminary investigation, future longitudinal studies of this type would benefit from the collection of maternal plasma and/or salivary measures at multiple time points, along with maternal behavioural ratings.

Chapter 8: Conclusions and future directions

8.1 Introduction

Based on the existing neuroimaging literature in child and adolescent antisocial behaviour this thesis aimed to use DT-MRI to examine, for the first time, white matter integrity in Conduct disorder (CD). Specifically, I hypothesised that boys with CD would have significant differences in white matter integrity, compared to typically developing children, within brain networks underlying emotion and social behaviour. I expected a reduction in white matter integrity, based on a single previous study that used DT-MRI to examine the UF in an antisocial population (Craig et al, 2009). In contrast to that adult study, I found increased white matter integrity in the UF. Based on the findings of these studies, I subsequently conducted a preliminary investigation to assess whether, in the general population, prenatal stress exposure modulates development of these white matter tracts.

This chapter summarises the main findings of the three experimental chapters that constitute the thesis, and then outlines the strengths, limitations, and overall contribution of these studies to the field of Conduct disorder research. Finally, it details how future research in the field could build on these studies.

8.2 Summary of main findings

The first experiment in the thesis, reported in Chapter 5, constituted the first DT-MRI study of adolescents with CD. Based on evidence showing abnormal limbic-

prefrontal ‘connectivity’ in antisocial adults (Craig, Catani et al. 2009; Motzkin, Newman et al. 2011; Sundram, Deeley et al. 2012), I applied DT-MRI tractography to examine the integrity of the uncinate fasciculus (UF) limbic-prefrontal white matter tract, and two non-limbic ‘control tracts’, in a group of boys with CD and a healthy comparison group.

Compared to healthy controls, adolescent boys with CD showed significantly *increased*, rather than decreased, white matter microstructural integrity of the left UF - but not the control tracts, as indexed by greater fractional anisotropy (FA). FA is understood to reflect inter- and intracellular white matter microstructural properties, including myelination, and axonal diameter and organisation (Beaulieu 2009). While this finding was in the opposite direction to what was predicted, it nevertheless suggested that between group difference specific white matter tracts within the limbic-prefrontal brain network may contribute to the social, emotional, and behavioural difficulties exhibited by antisocial children (possible reasons for this opposite result are discussed under ‘Limitations’ below). Although the integrity of the UF was not associated with behavioural variables in the CD group, this may have been the result of small sample size. Finally, *post hoc* analysis found a significant between group difference in the relationship between UF integrity and age. In healthy controls D_{perp} of the left UF was negatively correlated with age. This is the pattern reported in typical development (see Lebel et al., 2008). However, in CD this pattern was not observed. This might indicate an early acceleration of maturational processes (e.g. myelination) within the UF tract of boys with CD. However, as I did not specifically quantify myelin content, it is not possible to determine the basis for

the between group difference in maturation of D_{perp}. Therefore, taken together, Chapter 5 provided the first evidence that adolescents with CD significantly differ from controls in the microstructural anatomy, and possibly the maturation, of the uncinate fasciculus.

Chapter 6 aimed to extend the findings of the *a priori* tractography study, by comparing whole brain white matter integrity in the same cohort of boys studied in Chapter 5. Using the semi-automated voxel based analysis software Tract-Based Spatial Statistics (TBSS), I reported increased FA in regions corresponding to the fronto-cerebellar brain circuit, which connects the prefrontal cortex and cerebellar regions to the brainstem and spinal cord. These tracts are known to be involved in aggression and affective processing, as well as being integral to motor function. *Post hoc* analysis found that in several of these tracts FA was significantly associated with antisocial behaviour measures. Similar to the previous study, these correlations were found in the whole sample but not in the CD and control groups alone. However, the correlations were normally distributed among the two groups, and there were no outlier effects detected. This suggests a dimensional relationship between tract integrity and antisocial behaviour in young people, and one that is not restricted to CD individuals alone. Last, a third analysis showed significant age related increases in FA of 3 brain regions (bilateral middle and superior cerebellar peduncles and corticospinal tract) among healthy controls, but not in the CD group. While the correlations were not significantly different between groups, the different associations with age reinforce the previous chapter's suggestion that there may be atypical white

matter maturation occurring in CD. However, only further longitudinal investigations can verify whether this is the case.

Therefore, the two DT-MRI studies reported in Chapter 5 and 6 collectively provide initial evidence that there are significant differences in white matter integrity between adolescent males with CD and their typically developing peers, and that in all young males variation in white matter tract anatomy may be associated with degree of antisocial behaviour. Further, the whole brain investigation extended the tractography study, showing the white matter differences to be more widespread than initially thought. However, it is not clear from these studies how the white matter differences arise, or whether they predict behavioural outcomes.

Therefore, in order to address the question of how these differences may arise I conducted another investigation, which is reported in Chapter 7 of the thesis. This preliminary study examined whether in healthy children limbic-prefrontal white matter integrity is associated with exposure to maternal stress or *in utero* cortisol during the prenatal period. This study used DT-MRI tractography to assess FA and D_{perp} of the UF, and a non-limbic control tract, using similar methods to those followed in Chapter 5. I reported a positive correlation between maternal antenatal stressful life events and white matter integrity of their child's right UF. Conversely, in utero cortisol was negatively correlated with integrity of children's left UF, but the precise explanation for this is not clear. There were no significant correlations between prenatal factors and DT-MRI indices of the control tract, nor were other sociodemographic and obstetric

variables associated with integrity of either tract. These findings suggest that in humans maternal prenatal stress modulates the brain development of healthy children; and this includes white matter tracts that form part of the brain networks underlying social behaviour.

8.3 Contribution to knowledge

The studies within this thesis make a contribution to, first, current knowledge about white matter structure in children with antisocial behaviour. Only one previous neuroimaging study has reported on white matter neuroanatomy in CD, and this group used structural MRI to examine white matter concentration. These authors reported white matter concentration to be reduced in frontal and temporal areas, and increased in the middle frontal gyrus (De Brito, McCrory et al. 2011). However, structural MRI can provide only limited information about the integrity of specific tracts (Kubicki, Westin et al. 2006). Therefore, my studies are the first to use DT-MRI in CD, and provide a more detailed picture of the white matter architecture in CD than has previously been known.

Second, the thesis makes a contribution to the literature pertaining to perinatal psychiatry. It constitutes a novel study by virtue of its recruitment of participants in foetal life. Only one study to date has examined the effects of prenatal factors with neuroimaging, and this reported only structural grey matter findings (Buss, Davis et al. 2010). My study is the first to assess the limbic-prefrontal white matter microstructural integrity of children in whom prenatal stress and *in utero*

cortisol levels are known, thus providing important (albeit preliminary) information about the neurodevelopmental outcomes associated with prenatal mood and endocrinology.

Therefore, my research extends the existing knowledge base within the field of developmental neurobiology, and it provides a foundation that future research can now build upon.

8.4 Limitations

While the constituent studies within the thesis make a contribution to research in their respective fields, it is important to acknowledge the limitations with the studies.

First, I did not find significant correlations between uncinate fasciculus integrity and antisocial behaviour measures in any of the three studies. This may have resulted from a lack of power due to small sample sizes; larger sample sizes may have revealed significant associations between these measures. More specifically, in Chapter 5, a larger sample size may have revealed significant differences between white matter measures of boys with high levels versus low levels of CU traits. This may have then revealed correlations between DT-MRI measures and behavioural scores within the CD+CU group, enabling an examination of how these related to PCL-YV total and factor scores. This could have been of potential interest as children with higher CU traits and PCL-YV

scores are at increased risk for more severe and chronic antisocial behaviour (Frick and Marsee 2006). Similarly, early-onset CD is similarly associated with greater levels of delinquency, aggression and violence (Kazdin 1995; Jeglum Bartusch, Lynam et al. 1997) than a later onset. However, in this study age of onset was not associated with increased anatomical abnormality. Whilst this finding is consistent with recent studies (Passamonti, Fairchild et al. 2010; Fairchild, Passamonti et al. 2011), future studies would benefit from examining this issue within a larger cohort.

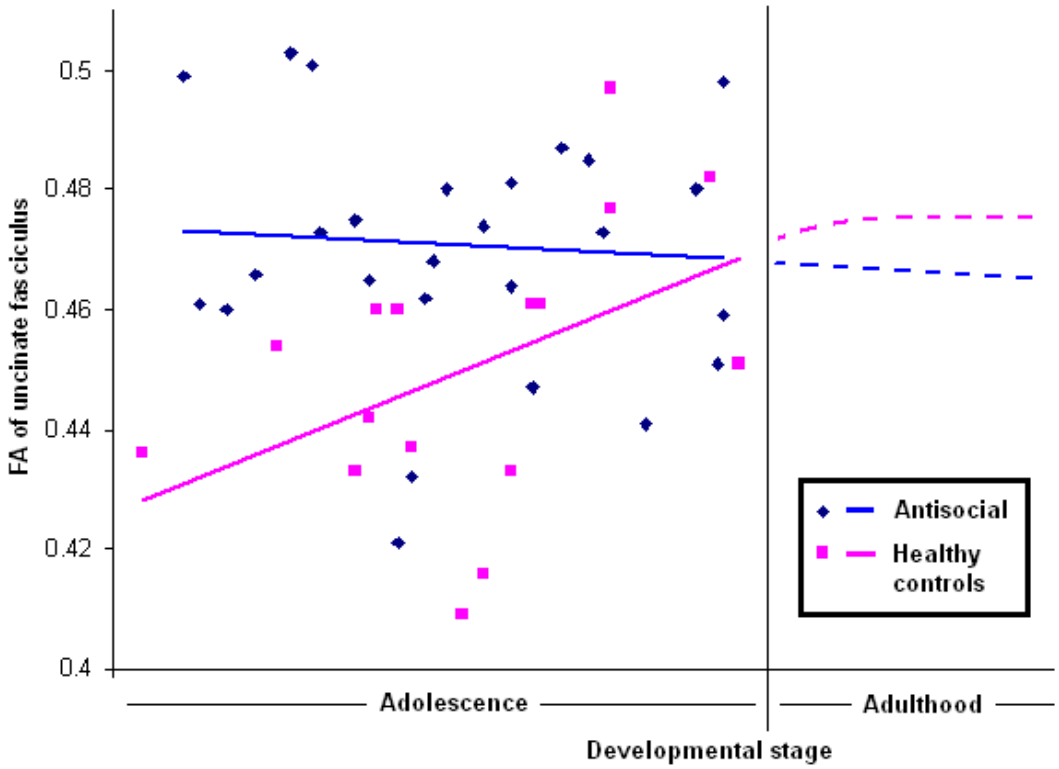
Second, the study of antenatal maternal stress in Chapter 7 may have suffered from floor effects, as there was limited variance in (and relatively low levels of) the number of stressful life events mothers had experienced during pregnancy (1-4 events from a possible 26). Nevertheless, I found a significant association between number of self reported stressors and DT-MRI indices despite these low stress levels. Thus, it can be argued that if such low stress levels produce such a significant effect, then even higher prenatal stress levels may be associated with even more striking associations with UF integrity.

Third, the group of children with CD (reported in Chapters 5 and 6) were recruited from, predominantly, non-forensic community samples. While these children had carried out extremely serious offences (e.g. robbery, grievous bodily harm, and sexual assault), I would probably have identified children with more severe antisocial behaviour had I have recruited from juvenile detention centres, and other forensic settings. In turn, this may have highlighted greater differences in white matter indices between groups, which may have better

associated with behavioural measures. However, recruiting from such settings presents significant practical and ethical difficulties. Therefore, as a first step, for these studies to develop proof of concept, I selected participants with the highest occurrence of antisocial behaviour possible in community samples. However, one of the strengths of recruiting from community samples is that it makes these research findings more generalisable to the wider population of most children with CD, whose antisocial behaviour – while significant – does not generally cross the threshold for incarceration within the criminal justice system. Similarly, another strength of the study lies in its recruitment of a healthy control group who closely resemble the CD group in many respects, such as age, IQ, and other socio-demographic factors. This helps to address research issues surrounding, for example, the confounding effects of hyperactivity, and drug and alcohol use, which are common among antisocial samples.

A further issue for consideration, is that the UF difference reported in CD in Chapter 5 was in the opposite direction (i.e. increased rather than decreased FA) to that previously reported in adults with antisocial behaviour (Craig, Catani et al. 2009). It is not clear what the significance of the opposing nature of these two studies may be, but it can be speculated to be related to an initial acceleration of white matter integrity in childhood, which plateaus later in development. Thus, where healthy children initially show a lower level of white matter integrity but then follow the typically maturational pattern (i.e. FA increases with age; D_{perp} decreases with age; Lebel, Walker et al. 2008) – come adulthood their UF integrity may be greater than the antisocial group (see Figure 8.1).

Figure 8.1: Proposed developmental trajectory of fractional anisotropy of the uncinate fasciculus with age



FA – fractional anisotropy. The left side of the graph shows increased FA of the uncinate fasciculus (UF) in antisocial adolescents compared to healthy controls, as reported in the DT-MRI tractography study in Chapter 5. The right side of the graph extrapolates my results, and depicts the proposed trajectory of UF FA into adulthood, providing an explanation for the results of Craig et al. (2009), where antisocial adults have reduced FA compared to healthy controls. In typical development UF FA plateaus after around age 30 (see Lebel et al. 2008).

The idea that increased FA in CD could indicate an accelerated rate of white matter development in these adolescents is consistent with similar patterns of white matter maturation observed in other specific neurodevelopmental disorders. For instance, structural MRI studies show that compared to neurotypical controls, people with autistic spectrum conditions (ASC) show increased white matter volume in early childhood, but the opposite pattern (i.e. decreased volume) during adolescence (Courchesne, Karns et al. 2001). This suggests that in ASC there may be an initial acceleration of white matter maturation followed by later reduced growth (Courchesne 2004). This early acceleration also corresponds with DT-MRI research showing FA to be increased in specific brain regions (e.g. right inferior frontal gyrus and left occipital lobe (Cheung, Chua et al. 2009); corpus callosum and cingulum (Weinstein, Ben-Sira et al. 2010)) in ASC. Taken together, these studies provide a plausible explanation for the incongruity between my study's finding of enhanced FA of the uncinate fasciculus in children with CD, and the reduced FA previously reported in antisocial adults (Craig, Catani et al. 2009). However, it is only possible to confirm this hypothesis through future longitudinal studies.

A further important point is that while my two studies of childhood antisocial behaviour both found significantly increased FA in CD as compared to healthy controls, they did not identify differences within the same tracts. In other words, TBSS analysis did not identify between group differences in limbic tracts such as the UF. The reason for this may arise from the nature of TBSS analysis. The software used to perform the voxel-wise statistics is called Randomise, and this sequentially compares every voxel to its corresponding voxel within each

participant's brain. Thus, differences seen using TBSS can be viewed as more gross than tractography, as differences need to withstand multiple statistical comparisons. In contrast, tractography can be used to detect more subtle differences in specific tracts. Thus, while there is increased FA in the UF compared to the non-limbic control tracts (as reported in Chapter 5), these may not be as pronounced as the increases in FA found in the projection tracts seen in Chapter 6. It is for this reason that my follow-up study will perform tractography on the tracts identified as having greater FA in CD using TBSS analysis.

A general methodological consideration should be made with regard to the tractography analysis used in the studies reported in Chapters 5 and 7. Tractography, in comparison to TBSS (used in the study in Chapter 6), is an operator reliant method. Thus, it could be argued that, as such, it is open to operator bias. Therefore, in order to limit the possible influence of bias on the analysis, I performed the analyses blind to clinical groupings and other bio-behavioural factors. Further, before performing any of the tractography dissections I received full reliability training by experienced tractographers. Therefore, I ensured that the results of the tractography studies were robust and replicable.

Another point of importance is that the effect size on which the power calculation for study 1 was based was large (2; see p.100), thus revealing only the strongest results. Consequently, other important findings may have failed to reach significance and this may have resulted in type II errors.

A general limitation that should be mentioned is that my studies of CD only recruited adolescent males. Thus, the findings cannot be generalized to females with CD. However, there are a number of reasons that many studies of CD, including my two studies within this thesis, have recruited only males. First, given the higher proportion of males to females presenting with CD at all ages (Moffitt and Caspi 2001), the recruitment of females is more difficult. Second, due to sex differences it would be important to examine males and females separately with regard to the effects of, for example, aging – and this would require recruitment of a much larger sample size. Sex differences to be considered include the finding that boys show a more protracted pattern of white matter development in most tracts compared to females (Asato, Terwilliger et al. 2010), and that in females white matter maturation of most tracts occurs by an earlier age (*ibid*). Also, by adulthood, males have a greater total white matter volume proportionate to overall brain volume (Schmithorst, Holland et al. 2008). The effects of hormones, including sex hormones during puberty, may influence brain development (Geidd, Clasen et al. 2006); so future studies including females should assess pubertal stage (e.g. by the inclusion of Tanner scales).

Last, the studies within the thesis were not able to assess the potential effects of neglect, abuse, or childhood trauma on behaviour and neurodevelopment. There is increasing evidence that adverse life events such as these can have long lasting effects of white matter structure. For example, children exposed to early socio-emotional deprivation exhibit significant differences in UF integrity

as compared to non-deprived children (Eluvathingal, Chugani et al. 2006). Similarly, adults who reported having been verbally abused by their parents in childhood show reduced FA in two 'limbic' white matter tracts (cingulum and fornix) and also the arcuate fasciculus (Choi, Jeong et al. 2009). While, in contrast to my findings, these studies show reduced FA in deprived/abused childhood, neither study included adolescents – which may reduce the generalisability of those data to my findings. Further, a structural MRI study recently reported larger white matter volumes in physically abused children in the right posterior cingulate, and bilateral cerebellum and prefrontal cortex. Also, greater volume was associated with increased behavioural problems (Hanson, Chung et al. 2010). Two of these regions (the cerebellum and prefrontal cortex) are the same as those I found to have significant differences in white matter integrity of CD youngsters (see Chapter 5 and 6); however, whether white matter volume is correlated with greater FA is not evident from these studies. Thus, while it is not clear what effect different types of childhood maltreatment have on neurodevelopment, it is important that these be considered in future neuroimaging studies of children with, for example, inclusion of a standardized screening measure such as the Childhood Trauma Questionnaire (Bernstein and Fink 1997).

8.5 Future directions

Following from the research presented in this thesis, there are a number of further analyses that I would like to carry out in order to answer further

questions. First, in Chapter 6 I presented tractography data pertaining to the UF tract. While this was a hypothesis driven investigation, it would be useful to also examine the microstructural integrity of other white matter tracts using this method (i.e. use tractography on whole brain white matter). Similarly, having shown (using TBSS) in Chapter 6 that a number of projection tracts have greater FA in CD than in controls, it is a necessary next step to use tractography to examine these tracts in more detail. While FA values were extracted from the TBSS analysis in Chapter 6, tractography dissections are able to provide more detailed microstructural data. With this data it would be possible to determine, not only which the white matter tracts/regions are that differ most between typical and CD children, but also which tracts associate more with particular antisocial behaviours. These may not be the same tracts, as not all behaviours that constitute a diagnosis of CD will necessarily be associated with a particular fibre bundle as those needed to simply meet diagnostic criteria for the disorder. For instance, a diagnosis of CD can be made where children meet 3 or more criteria from a possible 15, which include both overt (e.g. violence) and covert (e.g. lying) behaviours. Thus, it may be that a particular category of antisocial behaviour corresponds to limbic-prefrontal white matter, whereas different behaviours associate with other tracts or brain regions. Therefore, future research would benefit from performing analyses at the symptom level, rather than using the proxy measure of diagnostic classification alone.

In addition to examining individual symptoms against white matter integrity, it would be interesting to examine different subgroups of antisocial adolescents.

It is clear that CD is a heterogeneous disorder, and different methods of subtyping affected children have been proposed (Frick and Ellis 1999). Two of these groupings were examined in Chapter 5, namely the presence of psychopathic/callous-unemotional traits, and age of onset. The different associated risks and outcomes for these subtypes confirm that fractionating the disorder is essential. This may aid in devising effective interventions, predicting outcomes, and assessing causative factors. One method of examining subtypes would be through the use of new machine learning techniques, such as support vector machines (SVM). These machines first require training with examples of, for instance, CD and non-CD neuroimaging data. After creating a model, the software is then able to classify new data as belonging to one or other of the diagnostic categories it has been trained with. Thus, SVM could potentially be used to explore whether based on neuroanatomical features alone, CD subtypes such as with/without CU traits, early- vs adolescent-onset, comorbid with ADHD, etc. can be differentiated.

A further analysis I propose to conduct will assess grey matter volume within limbic-prefrontal brain regions, in order to assess the relationship between this and UF integrity in both of my study cohorts. It is currently not known whether differences in grey matter relate to those in white matter integrity in these populations. The basis for planning this investigation lies with evidence of reduced grey matter volume in the prefrontal cortex, temporal lobe, and limbic structures in CD (e.g. Huebner, Vloet et al. 2008), and in the prefrontal and temporal lobes of children prenatally exposed to high rates of maternal anxiety

(Buss, Davis et al. 2010). Thus, this investigation would address the current lack of data.

There are several further investigations that future studies would benefit from including in their protocols. First, it will be necessary to collect DNA in order to examine key genetic markers that may be associated with the white matter findings reported in my studies. For example, a recent study has reported on several genes that influence white matter integrity (Kohannim, Jahanshad et al. 2012); however, it is not known whether similar genetic modulation of white matter occurs in CD. Further, it would be interesting to examine whether genes associated with CD are associated with the white matter differences identified in my studies. Finally, several genes implicated in CD that influence hormonal and neurotransmitter activity would be important targets for future studies to combine with white matter neuroimaging (e.g. Comings, Chen et al. 1999; Rowe, Stever et al. 2001; Cadoret, Langbehn et al. 2003).

Last, an important future study would be to specifically recruit families whose child is at risk of developing antisocial behaviour. The child may be at risk because they present with multiple known environmental, familial, and social factors that are associated with CD (e.g. parental substance use, low socio-economic status, ineffective parenting, trauma, abuse, neglect, etc. (Matthys and Lockman 2010)), and/or have a sibling with a diagnosis of CD. Mothers could be recruited during pregnancy (using similar methods to those reported in my study in Chapter 7) and their child assessed with neuroimaging during infancy and/or childhood. Such a longitudinal study could identify whether the

white matter differences I observed in adolescents with CD are present at an earlier developmental stage (i.e. whether there is an early difference in white matter microstructural integrity in 'at risk' children as compared to typical children). These data would assist in implementing early intervention strategies.

8.6 Conclusion

Taken together the three studies constituting this thesis tentatively support the hypothesis that childhood antisocial behaviour is associated with differences in the integrity of white matter in brain networks associated with social and emotional behaviour, namely the limbic-prefrontal and fronto-cerebellar circuits. Further, my data suggest that exposure to antenatal maternal stress may be one factor that modulates fronto-limbic 'connectivity'. This modulation appears to be specific to the UF, which is the same tract showing increased white matter integrity in adolescents with antisocial behaviour, compared to typically developing youngsters. However, these studies are not able to determine the mechanism behind the white matter changes observed. For example, limitations with current DT-MRI techniques make it impossible to deduce whether the UF is more densely myelinated, or whether the fibres have greater packing density, or greater diameter (Beaulieu 2009; Paus 2010). Future studies could elucidate this issue by, for example, including an imaging sequence that quantifies myelin content (e.g. myelin mapping; Deoni, Mercure et al. 2011). Finally, although my studies link prenatal events to white matter

neurodevelopmental outcomes, these data cannot identify whether these differences persist, or if they associate with child behavioural outcomes. In order to examine these issues, longitudinal follow-up of children from this study would be necessary. Nevertheless, these studies provide a foundation upon which future research can further develop.

Appendices

Appendix 1: Table of human studies of prenatal stress and offspring neurodevelopmental outcomes

Author	Date	Maternal measure	Prenatal time point	Postnatal time point	Child behavioural or neurological outcome
Austin et al	(2005)	Anxiety (trait)	32 weeks	4 & 6 months	Difficult temperament (SITQ)
Barker & Maughan	(2009)	Anxiety	29-40 weeks	4-13 years	Persistent conduct problems (SDQ)
Bergman et al	(2007)	Stressful life events during pregnancy	retrospective	10-19 months	Increased fear reactivity (LabTAB), reduced cognitive development (BSID-II)
Buss et al	(2010)	Pregnancy related anxiety	19 weeks	6-9 years	Reduced grey matter density in prefrontal cortex
Davis et al	(2007)	Cortisol	18-32 weeks	2 months	Increased negative reactivity (IBQ)

		Depression			
DiPietro et al	(2010)	Pregnancy related stress	24-38 weeks	Neonatal	Greater neurological maturation (increased motor reflexes)
De Weerth et al	(2003)	Cortisol	36 weeks	1-20 weeks	Increased difficult behaviour (ICQ)
Gutteling et al	(2005b)	Perceived stress	15-38 weeks	27 months	Increased behavioural problems (CBCL)
Hay et al	(2010)	Depression	14-36 weeks	11 & 16 years	Antisocial behaviour (CAPA)
Huizinck et al	(2002)	Perceived stress	15-38 weeks	3 months	Increased behaviour problems
O'Connor et al	(2002b)	Anxiety	32 weeks	4 years	Increased behavioural and emotional problems (SDQ)
O'Connor et al	(2003)	Anxiety	32 weeks	4 and 7 years	Increased behavioural and emotional problems (SDQ)

Rice et al	(2010)	Stress	31-40 weeks	4-10 years	Increased conduct problems (SDQ)
Van den Bergh el al	(2004)	Anxiety (state)	12-22 weeks	8-9 years	Externalising problems, ADHD, anxiety (CBCL)
Van den Bergh et al	(2005)	Anxiety (state)	12-22 weeks	14-15 years	Increased impulsivity; reduced vocabulary and block design IQ subscores

BSID-II – Bayley Scales of Infant Development (Bayley 1993); CAPA – Child and Adolescent Psychiatric Assessment (Angold and Costello 2000); CBCL – Child Behaviour Checklist (Achenbach 1991); IBQ – Infant Behavior Questionnaire (Gartstein and Rothbart 2003); ICQ – Infant Characteristics Questionnaire (Bates, Freeland et al. 1979); LabTAB – Laboratory Temperament Assessment Battery (Goldsmith and Rothbart 1999); STIQ – Short Infant Temperament Questionnaire (Sanson, Prior et al. 1987); SDQ – Strengths and Difficulties Questionnaire (Goodman, Ford et al. 2000)

Appendix 2: Ethical approval letter 1 – Study 1

The Joint South London and Maudsley and the Institute of Psychiatry NHS Research Ethics Committee
1ST Floor
Camberwell Building
94 Denmark Hill
London SE5 9RS

Telephone: 020 3299 5033

20 December 2006

Prof Declan Murphy
Po 50 Institute of Psychiatry
De Crespigny Park
London SE5 8AF

Dear Prof Murphy

Study titles: The neural correlates of facial emotional processing in psychopaths: an fMRI study
REC reference: 243/00
Amendment date: 1 December 2006

The above amendment was reviewed at the meeting of the Sub-Committee of the Research Ethics Committee held on 15 December 2006.

Ethical opinion

The members of the Committee present gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

- Notice of substantial amendment form dated 18 October 2006
- Supporting documents: Cover letter to committee 18.10.06; letter to parent/young person 17.10.06; parent information sheet and consent form 17.10.06; young person information sheet and consent form 17.10.06
- Email dated 1 December 2006

Management approval

All investigators and research collaborators in the NHS should notify the R&D Department for the relevant NHS care organisation of this amendment and check whether it affects local management approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

243/00:	**Please quote this number on all correspondence**

Yours sincerely

Jenny Liebscher
Committee Administrator

E-mail: ethics.office@iop.kcl.ac.uk

Appendix 3: Ethical approval letter 2 – Study 1

The Joint South London and Maudsley and The Institute of Psychiatry NHS Research Ethics Committee

South London REC Office (2)
1st Floor, Camberwell Building
94 Denmark Hill
London
SE5 9RS

25 August 2009

Sagari Sarker
Institute of Psychiatry at the Maudsley
Section of Brain Maturation
Division of Psychological Medicine
PO Box 50
De Crespigny Park
Denmark Hill
London
SE5 8AF

Dear Sagari Sarkar

Study title: **Brain Structure and Face Perception**

REC reference: **243/00**
Amendment number: **1**
Amendment date: **16 July 2009**

The above amendment was reviewed at the meeting of the Sub-Committee held on 21 August 2009.

Ethical opinion

The members of the Committee taking part in the review gave a favourable ethical opinion of the amendment on the basis described in the notice of amendment form and supporting documentation.

Approved documents

The documents reviewed and approved at the meeting were:

Document	Version	Date
Original adult study ethical application signed by new Chief Investigator	1	
Original addendum request	1	18 October 2006
New Chief Investigator CV	1	
Patient Information Sheet	2	16 July 2009
Parent Information Sheet	2	16 July 2009

Recruitment Advertisement	1	16 July 2009

R&D approval

All investigators and research collaborators in the NHS should notify the R&D office for the relevant NHS care organisation of this amendment and check whether it affects R&D approval of the research.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

243/00:	**Please quote this number on all correspondence**

Yours sincerely

Chris Ward
Committee Co-ordinator

E-mail: chris.ward@kch.nhs.uk

Copy to: *Mrs Gill Lambert*
[R&D office for NHS care organisation at lead site]

Appendix 4(a): Patient Information Sheet – Study 1

Institute of Psychiatry
at The Maudsley

Section of Brain Maturation
Division of
Psychological Medicine

Professor Declan Murphy

PO Box 50
De Crespigny Park
Denmark Hill
London SE5 8AF

KING'S College LONDON Founded 1829
University of London

Information sheet for parents

Brain structure and face perception

PRINCIPAL INVESTIGATOR: Dr. Quinton Deeley

We are asking you to help us by allowing your son to participate in the following study:

- We are studying face perception, and we want a wide variety of people to take part.
- The study might not help your son, but it may help other people. If you do not want him to take part in the study, that is all right and it will not affect his future schooling or healthcare.
- Your son can stop taking part in the study any time. If he is unhappy about any part of what is going on, he can let us know and we will stop immediately.

What will happen if your son takes part?

- We will meet with your son and take a medical and psychiatric history and perform some simple tests. We will also administer a number of questionnaires to help find out how your son has been feeling in himself recently.

Psychological Investigations:

Your son will be given some simple tests of his ability to use words and a test of his memory. Another task involves showing him some pictures of people's faces on a computer screen and he will have to press a button if it is a male face. We will also ask him to fill in a personality questionnaire. These tests take around 1½ hours, but we will space them out and give your son rests. Your son should not be upset if he can't do all these tests because nobody can do them all.

Brain Scanning:

The next thing we want to do is a MRI (Magnetic Resonance Imaging) scan. With the MRI, we can take pictures of the brain therefore your son will have to lie as still as possible so we can get the best pictures possible.

For the scan your son will lie in a large machine that looks a bit like a tube. In order to be scanned your son/daughter will lie down on a table, which slides into the tube. The tube will be open at both ends all the time.

Your son will simply be asked to rest for the duration of the scanning, which is around 45 minutes.
In the machine your son will hear loud noises, but this is nothing to worry about the machine will not hurt him at all.

Safety issues:

If your son has any **metal** pieces in his body then he **should not** go into the scanning machine. For example, he **must not have a scan if** he:

- has received **metal injuries to the eye** (caused by metal objects, for example by using a welder)
- has had **metallic objects (including clips)** inserted into his body during an operation
- has a **heart pacemaker**
- has ever received a **shotgun** injury

The radiographer will go over a list of possible risks with him before he goes into the scanner.
MRI scans do not involve any radiation.

There is a microphone inside the scanner so that your son can talk to us at any time he likes. If he feels slightly scared being in the scanning machine we can stop the scanning immediately. The other thing he might find uncomfortable is the noise the machine makes, but most people get used to it after a while.

In the unlikely event that we find anything unexpected on your son's brain scan we will contact his GP.

Remember that you and your son are free to withdraw from the study at any time without giving a reason. This will not affect his current or future medical treatment in any way

Thank you very much for reading this information. If you are worried about any aspect of this study please contact Dr Quinton Deeley on (020) 7848 0984, or write us at the Section of Brain Maturation, PO50, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

Appendix 4(b): Consent form – Study 1

CONSENT FORM

Parent

Brain structure and face perception

I, _____
(Name in capitals)

The parent/guardian of _____
(Child's name in capitals)

Agree for my son to take part in the project as explained to me by

_____
(Researcher's name in capitals)

I have read the attached information sheet, having had the chance to discuss the matter with the investigating doctor, and I am willing to participate in the research. Please tick the items on the check list.

The study organisers have invited my son to take part in this research

- I have read what is in the leaflet about the research. I have a copy of the leaflet to keep.
- I have had a chance to talk and ask questions about the study. I know how long it will take.
- I have been told about any tests or other checks my son might be given.
- I have been told there are possible risks.
- I understand my son should not take part in more than one study at a time.
- I know that the local South London and Maudsley NHS Trust Research Ethics Committee has reviewed the study.
- I understand that personal information is strictly confidential: I know the only people who may see information about my son's part in the study are the research team.
- I consent for my son to be a subject in the study. No-one has put pressure on my son or me.
- I know that my son can stop taking part in the study at any time.
- I know that if there are any problems I can contact Professor Murphy or Dr Quinton Deeley on 0207 848 0984

Signed _____ (Parent)

Date _____

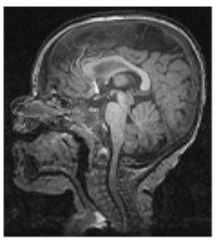
Appendix 5: Recruitment flyer – Study 1

Institute of Psychiatry

at The Maudsley

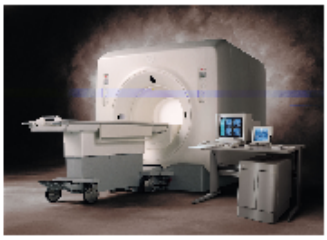
KING'S College LONDON
University of London

Would you like to help with a research study?



We are looking for boys between 12 and 18 years old to take part in a study of brain structure and face perception.

Participation involves a 2½ hour session at the Institute of Psychiatry in Camberwell. You will be given some simple computer tests, such as looking at some faces and identifying if they are male or female. You will also be asked to complete some questionnaires. Finally, we will use MRI scanning to look at your brain structure and function.



MRI scanning is safe to use and does not involve any radiation. We hope to learn more about which areas of the brain are involved in the ability to recognise emotion in faces.

We will reimburse you £50 for your time, and pay for your travel costs.

If you would like to find out more or volunteer to take part, please get in touch with the study co-ordinator on 0207 848 0943 or 07548 950016 or by email to facestudy@hotmail.co.uk

SLaM/IoP NHS Ethics Committee Reference Number: 243/00

Appendix 6: MRI Safety form

South London and Maudsley NHS Foundation Trust

SAFETY QUESTIONNAIRE FOR MRI

SURNAME..... **FIRST NAMES** .....

D.O.B..... **HOME TEL:** .....

ADDRESS:.....

.....

(Please circle correct response)

1. Have you had any Scans or X-rays here before? MRI / CT / X-rays / None
2. Do you have a pacemaker or artificial heart valve fitted? Y/N
   Any other heart or chest operations? Y/N
3. Have you had any operations on your head, ears or spine? Y/N
4. Have you had any operations where metal might have been inserted into your body? Y/N
   If 'Y', please give details .....
   Have you had any other operations? Y/N
   If 'Y', please give details .....
5. Do you have any foreign metallic bodies in your eyes? Y/N
   Have you done any welding or metalwork? Y/N
   Do you have any shrapnel in your body? Y/N
6. Do you have any of the following:

Dentures, dental plates or bridges	Y/N	False limb, calliper or brace	Y/N
Tattoos / metallic make-up	Y/N	Hearing aid or ear implant	Y/N
Body Piercings	Y/N		

   Any implanted device that is electrically, magnetically or mechanically activated? Y/N
7. Do you have a history of (a) Seizures Y/N
   (b) Diabetes Y/N
   (c) Allergic reaction to drugs? Y/N
   please state which drugs .....
8. Is there any chance that you may be pregnant? Y/N
9. Do you have a history of any problems with your heart or arteries? Y/N
10. Do you have any history of kidney problems Y/N
11. Are you able to lie flat without becoming breathless? Y/N
12. How much do you weigh? ....

The reverse of this questionnaire gives some background information and reasons why your brain scan may help further our understanding of the human brain. Please read this carefully.

Client Signature.....**Date** .....

Authorised CNS Signatory .....

Include in Database **Yes** ☐ **No** ☐ **PTO**

Appendix 7: Fax template used to obtain surgical notes

Institute of Psychiatry
at The Maudsley

Section of Brain Maturation
Division of Psychological Medicine
Institute of Psychiatry

Tel : 0207 848 0943
Mobile: 079313 53234
Fax : 0207 848 0650
Email : sagari.sarkar@kcl.ac.uk

KING'S College LONDON Founded 1829
University of London

FAO XXXXXX
Access to Medical Records
XXX Hospital
Fax: XXXX XXXXXXX

Dear XXXXXX,

RE: XXXXXXX, XXXXXX DOB - XX.XX.XX Request for surgical notes

Further to our telephone conversation today, I am writing to request surgical notes for this patient.

Mr. XXXXX is due to undergo MRI scanning at the Institute of Psychiatry. In order to be deemed safe to scan we must ensure patients with a history of surgery have had no metallic clips, etc, left inside their bodies.

If you require any further information please do not hesitate to contact me.

Best wishes,

Sagari Sarkar
Study Coordinator

Appendix 8: Strengths and Difficulties Questionnaire – parent report

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Appendix 8: Strengths and Difficulties Questionnaire – self-report

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Appendix 9: K-SADS-PL Conduct disorder interview

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Appendix 10: Antisocial Process Screening Device – parent report

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CONTENT REMOVED FOR COPYRIGHT REASONS

Appendix 10: Antisocial Process Screening Device – self-report

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CONTENT REMOVED FOR COPYRIGHT REASONS

Appendix 11: PCL-YV criteria

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Appendix 12: Ethnicity screening categories

South London and Maudsley NHS Trust

Ethnicity Classification Categories and Codes

White		Asian or Asian British		Mixed background	
British	A	Indian/British Indian	H	White and Black Caribbean	D
Irish	B	Pakistani/British Pakistani	J	White and Black African	E
English	CA	Bangladesh/white Bangladeshi	K	White and Asian	F
Scottish	CB	Mixed Asian	LA	Black and Asian	GA
Welsh	CC	East African Asian	LD	Black and Chinese	GB
Cypriot (part not stated)	CE	Sri Lankan	LE	Black and White	GC
Greek	CF	Tamil	LF	Chinese and White	GD
Greek Cypriot	CG	Sinhalese	LG	Asian and Chinese	GE
Turkish	CH	British Asian	LH		
Turkish Cypriot	CJ	Caribbean Asian	LJ	Other Ethnic Groups	
Irish Traveller	CL	Other Asian unspecified	LK	Chinese	R
Traveller	CM			Vietnamese	SA
Gypsy/Romany	CM	Black or Black British		Japanese	SB
All former USSR republics	CQ	Caribbean	M	Filipino	SC
Kosovan	CR	Somali	PA	Malaysian	SD
Albanian	CS	Nigerian	PC	Middle eastern	SF

Bosnian	**CT**	**Black British**	**PD**	**Arab**	**SG**
Croatian	**CU**	**Sudanese**	**PH**	**Iranian**	**SH**
Serbian	**CV**	**Angolan**	**PJ**	**Iraqi**	**SJ**
Portuguese	**C4**	**Eritrean**	**PK**	**Colombian**	**SK**
Kurdish	**C5**	**Ethiopian**	**PL**	**Ecuadorian**	**SL**
Other White unspecified	**C3**	**Ghanaian**	**PM**	**Other Latin American**	**SM**
		Algerian	**PP**	**Any Other Group**	**SE**
		Ugandan	**PQ**	**Not Stated**	**Z**
		Other African	**N**		
		Mixed Black	**PB**		
		Other Black unspecified	**PE**		

Appendix 13: Socio-economic status questionnaire

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Appendix 14: Edinburgh Handedness Inventory

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Appendix 15: Ethics approval letter – Study 2

Brent Medical Ethics Committee
Room 019, Level 7 Maternity Block
Northwick Park Hospital
Watford Road
Harrow
Middlesex
HA1 3UJ

Telephone: 020 8869 3805
Facsimile: 020 8869 5222

03 July 2009

Dr Quinton Deeley
Senior Lecturer in Social Behaviour and Neurodevelopment
Institute of Psychiatry, Kings College London
Institute of Psychiatry
de Crespigny Park
Denmark Hill
SE5 8AF

Dear Dr Deeley

Study Title:	**Antenatal maternal stress, in utero cortisol exposure, cognitive development and brain structure and function, in 6 year old children**
REC reference number:	**09/H0717/39**
Protocol number:	**1**

The Research Ethics Committee reviewed the above application at the meeting held on 29 June 2009. Thank you for attending to discuss the study.

Ethical opinion

In discussion, the Committee noted the following ethical issues.

1. The committee asked why the researchers were not contacting the GPs considering they were going to carry out MRI scans and requested that this should be done.
2. The committee asked how the researchers would follow up the mothers and/or children should they become worried at a later stage after the study.
3. The committee asked what support could be offered from within the team during the study.
4. The committee asked for clarification as to how the findings would be communicated to the parents - the response to questions A14 and A53 need to correlate. This information also needed to be added to the Participant Information Sheet (PIS)
5. The committee noted that the Sponsor Clinical trials Insurance was due to run out on 31 July 2009 and requested that an updated one be sent to the office when available.

Ms Sagari Sarkar was invited to join the meeting and the Chair welcomed her and asked for a brief explanation of the proposed study, her role in the project and the main ethical issues in her opinion. In addition the Chair informed them that a letter would be sent following the meeting, which would set out the Committee's discussion and any amendment required to the documentation.

Ms Sarkar explained that the participants had been identified from Queen Charlottes Hospital through another study and she was picking up the group as the children will now be 6 years old. She would be involved in the data collection and administering the questionnaires and a research assistant would be involved in the recruitment. The main ethical issues were related to the MRI scan which could possibly cause anxiety for the children. They would make the process tolerable and reassure them throughout. The neuropsychological tests were not new but could make participants feel anxious and the confidentiality issues around DNA samples.

A. The Chair asked whether consideration had been given to how the results would be

received after 3 years. Ms Sarkar replied that there were possible implications related to the findings causing stress and knowledge regarding adverse events. Ms Sarkar continued that they were looking at stress minimization during pregnancy and were expecting significant findings. The committee asked whether Ms Sarkar had considered how the mothers could interpret the findings and how they would look after the mothers and children should they may have linked an unforeseen interpretation of the result. Ms Sarkar replied that she would need to consider this further.

B. The Chair asked Ms Sarkar to register the new research assistant once recruited following the appropriate procedures. Ms Sarkar agreed to do this.
C. The Chair informed Ms Sarkar that they must inform the GPs especially as an MRI scan was being done. Ms Sarkar agreed to do this.
D. The committee asked what help was available within the study team should something happen during scanning or the neuropsychological tests. Ms Sarkar replied that a radiologist would be carrying out the scans and trained first aiders were available.

The Chair asked Ms Sarkar to step outside of the meeting room to enable further discussion by the committee. The committee commented that Ms Sarkar had satisfactorily answered the committee's queries however the additional conditions must be agreed.

The members of the Committee present gave a favourable ethical opinion of the above research on the basis described in the application form, protocol and supporting documentation, subject to the conditions specified below.

Ethical review of research sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

The favourable opinion applies to the following research site(s):

Research Site	Principal Investigator / Local Collaborator
Institute of Psychiatry - Centre for Neuroimaging Sciences, Kings College London.	Dr Quinton Deeley

Conditions of the favourable opinion

The favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission or approval must be obtained from each host organisation prior to the start of the study at the site concerned.

For NHS research sites only, management permission for research ("R&D approval") should be obtained from the relevant care organisation(s) in accordance with NHS research governance arrangements. Guidance on applying for NHS permission for research is available in the Integrated Research Application System or at http://www.rdforum.nhs.uk. Where the only involvement of the NHS organisation is as a Participant Identification Centre, management permission for research is not required but the R&D office should be notified of the study. Guidance should be sought from the R&D office where necessary.

Sponsors are not required to notify the Committee of approvals from host organisations.

The Committee gave a favourable opinion of the application (with additional conditions)

i. The researchers must inform the participants GP that they have been recruited to this study.
ii. On page 3 of the PIS the final sentence should be revised to read "In the unlikely event of us finding any unexpected abnormalities or you suffering from the process (physical or psychological) we will ask your permission to contact yours and/or your child's GP".
iii. Confirmation was required of how the results would be disseminated.

Please send the revised documents to the ethics office and ensure they have been updated with final version numbers and dates.

It is responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Approved documents

The documents reviewed and approved at the meeting were:

Document	*Version*	*Date*
Questionnaire: State Trait Anxiety	Spielberger 1968, 1997	
Questionnaire: Social Communication Checklist	Rutter et al 2003	
Questionnaire: Bryant Empathy Index	Bryant 1982	
Questionnaire: Fear Survey Schedule for Children-revised	Ollendick 1983	
Questionnaire: Kiddie SADS	Kaufman 1996	
Covering Letter		02 June 2009
Questionnaire: Penn State Worry Questionnaire	Meyer et al 1993	
Questionnaire: Edinburgh Postnatal Depression Scale	Cox et al 1987	
Questionnaire: Antisocial Process Screening Device	Frick & Hare 2001	
Questionnaire: Conners Parent Rating Scale	Conners 1997	
Covering Letter		01 June 2009
Protocol	1	01 June 2009
Investigator CV		01 June 2009
Application		01 June 2009
Letter from Funder		04 February 2009
Letter from sponsor		28 September 2008
CV for Ms Sagari Sarkar		01 June 2009
CV for Vivette Glover		01 June 2009
CV for Michael Craig		01 June 2009
Certificate of Insurance		01 August 2008
Participant Consent Form	1	25 February 2009
Participant Information Sheet	1	25 February 2009
Letter of invitation to participant	1	25 February 2009
Questionnaire descriptions	1	25 February 2009

Membership of the Committee

The members of the Ethics Committee who were present at the meeting are listed on the attached sheet.

Statement of compliance

The Committee is constituted in accordance with the Governance Arrangements for Research Ethics Committees (July 2001) and complies fully with the Standard Operating Procedures for Research Ethics Committees in the UK.

After ethical review

Now that you have completed the application process please visit the National Research Ethics Service website > After Review

You are invited to give your view of the service that you have received from the National Research Ethics Service and the application procedure. If you wish to make your views known please use the feedback form available on the website.

The attached document "After ethical review – guidance for researchers" gives detailed guidance on reporting requirements for studies with a favourable opinion, including:

- Notifying substantial amendments
- Adding new sites and investigators
- Progress and safety reports
- Notifying the end of the study

The NRES website also provides guidance on these topics, which is updated in the light of changes in reporting requirements or procedures.

We would also like to inform you that we consult regularly with stakeholders to improve our service. If you would like to join our Reference Group please email referencegroup@nres.npsa.nhs.uk.

09/H0717/39	Please quote this number on all correspondence

With the Committee's best wishes for the success of this project

Yours sincerely

Dr C Bernard Colaço
Chair

Email: Mona.Shah@nwlh.nhs.uk

Enclosures: *List of names and professions of members who were present at the meeting and those who submitted written comments*
"After ethical review – guidance for researchers" [SL-AR2 for other studies]

Copy to: Mrs. Gill Lambert
Research Governance/Clinical trials Facilitator
Institute of Psychiatry, Office P005
De Crespigny Park
Denmark Hill
London
SE5 8AF

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Appendix 16: Transcript for demonstration video

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MRI study video - transcript

When you arrive for your appointment you will be met at the CNS by the researchers.

Next, our radiographer will go through some safety questions with you to make sure it is safe for your child to be scanned, and for you to stay in the room with your child during the scanning.

You will be asked to remove all metal objects and jewellery from yourself and your child. We have lockers available for you to keep your things.

Next, you will both go into the MRI room and your child will be helped onto the bed of the scanner by our radiographer.

The radiographer will give your child some earplugs to protect from the noise of the scanner, and also some headphones so that your child can listen to the DVD they have chosen to watch during the scan.

There is a mirror over your child's head so that they can look out at the TV screen and into the room at all times.

When your child is comfortable and happy, the bed will slide a little way into the tube of the scanner to make sure your child's head is in the correct position within the scanner.

Your child can speak to the radiographer and the scanner can be stopped at any time.

Your child will need to lie as still as possible while the scanner takes pictures of their head.

Don't worry about the scanner noise your child will wear earplugs will greatly reduce the scanner sound.

After the scan your child will be helped out of the scanner.

After a break for lunch, we will start the second part of the session.

Here the researcher will ask your child to complete some puzzles and tests on a computer. These are simple and fun to do.

Finally we would like to observe you and your child solving a short puzzle together. The whole day should last no more than 4 hours.

Appendix 17: GP letter – Study 2

Version 2: 01.08.10 Brent REC code 09/H0717/39

Institute of Psychiatry
at The Maudsley

Section of Brain Maturation
Division of Psychological Medicine

Professor Declan Murphy
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KING'S College LONDON
Founded 1829
University of London

Dr.
ADDRESS

DATE

Re: Participation of your patient in Research Project at the Institute of Psychiatry, KCL

Dear Dr. XXXXXXXX,

This letter is to inform you that your patient:

Name: .....

Date of birth: .....

has agreed to participate in a research project at the Institute of Psychiatry, KCL, titled *'Pregnancy and child development: a brain imaging study'*. Participation will involve an MRI scan of the brain, neuropsychological testing, and the completion of several questionnaires.

We will further inform you only if there are any clinically significant results.

Please do not hesitate to contact us if you have any queries.

Yours sincerely,

Dr. Michael Craig
Principle Investigator

Appendix 18: Children's MRI training schedule

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Appendix 19: Children's sticker chart

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Appendix 20: Initial contact letter – Study 2

Version 2. 01/08/10 BRENT NHS REC REC number 09/H0717/39

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Imperial College London

Dear

Study Title: **Pregnancy and Child Development: A brain imaging study**
Ethics No: **09/H0717/39**

You were most helpful to us during our follow up study: "Hormones and child development (study number 06/Q0403/19).

You may remember coming to the Children's Out Patients at Hammersmith Hospital or the study centre at Cambridge University with your child for a half day of observation and interviews with Professor Melissa Hines's Research team. You may have attended up to three of these visits when your child was age 4, 5 and 6. Thank you again for your participation.

Professor Vivette Glover at Imperial College London is now collaborating with Dr Quinton Deeley at King's College London on another follow-up study similar to the one in which you have already participated. We are studying child development at age 5-7 and investigating whether this may be related to how a mother feels emotionally during pregnancy, or her levels of stress hormones. These were measured in our first study: 'Maternal and fetal stress hormone levels' (study number 2001/6197). We will also be looking at whether child brain structure or function relates to the mother's emotional status during pregnancy. We will investigate this by using MRI scanning, a research technique that is widely used with babies and children.

We would like to invite you and your child to attend a half-day session at our Kings College London campus. Of course, we would arrange a time convenient for you and your child, and provide you with door to door transport and meals during the day. We will contact you during January 2010, and hope that you will continue to participate. There is no obligation to take part in this follow-up even though you consented to participate in the previous parts of the study. However, your continued help with this project would be greatly appreciated. Should you move house during this period, it would help to know your new address and phone number.

If you require further information, please feel free to contact Fiona Rose-Clarke on 0207 594 2188 or email fiona.rose-clarke@kcl.ac.uk.

Sincerely,

Fiona Rose-Clarke
Research Co-ordinator
Queen Charlotte's Hospital
020 7594 2188

Professor Vivette Glover
Imperial College
Hammersmith Campus
020 7594 2136

Michael Craig
Principal Investigator
Institute of Psychiatry
Kings College London
020 7848 0943

Appendix 21: Patient Information Sheet – Study 2

Version 3. 01/08/2010 BRENT NHS REC REC number 09/H0717/39

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Division of
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Information sheet for parents

Pregnancy and child development: A brain imaging study

PRINCIPAL INVESTIGATOR: Dr. Michael Craig

You are being invited to take part in a research study. Before you decide, it is important for you to understand why the research is being done and what it will involve. Please take the time to read the following information carefully and discuss it with others if you wish. Ask us if there is anything that is not clear or if you would like more information. Take time to decide whether or not you wish to participate.

What is the purpose of this study?

- We are studying child development, and investigating whether this may be related to how a mother feels emotionally during pregnancy, or her levels of stress hormones. These were measured in our first study: 'Maternal and fetal stress hormone levels' (registration number 2001/6197). We will also be looking at whether child brain structure or function relates to emotion during a mother's pregnancy, and we will investigate this by using MRI scanning.

Why have you been chosen?

- You will remember that you were recruited by Vivette Glover's research team at Queen Charlotte's Hospital several years ago during your pregnancy, at the time of your amniocentesis. You agreed to return when your child was around 17 months old for the first follow-up study. You also agreed to take part in the study: 'Hormones and child development' (registration number 06/Q0403/19) when your child was 4 years old. You may also have returned when your child was aged around 5.

- This study is another follow-up study, although you are not obliged to take part if you do not want to. If you do not want you and/or your child to take part in this study, that is all right and it will not affect your future medical care.

- You can withdraw from the study at any time. If you are unhappy about any part of the research process, let us know and we will stop immediately and not use any of your or your child's information.

What will happen if you and your child take part?

- We will arrange a day convenient for you and your child to come in to the Institute of Psychiatry in Denmark Hill (*see attached map*). Before the visit we will send you some questionnaires for you to complete about your child's behaviour and feelings. We will include a collection tube for your child to provide a saliva sample on the day of the visit (see 'Saliva samples' below). The appointment will last between 2 and 2½ hours, with as many breaks as are required.

- On the day you come in, you and your child will be met by our researcher, who will be happy to answer any questions you have. You will both be taken into a comfortable interview room and your child will be asked to answer some questionnaires that will be read to him/her by the researcher. These will ask simple questions (for example, about how he/she is feeling and his /her thoughts about everyday things).

- After this, your child will be asked to perform some simple tests, shown on a laptop computer screen. Your child will be asked to press a button to pictures or symbols on the screen as part of tests of his/her attention and memory. This first part of the day will take around one hour, and your child is able to take breaks whenever he/she needs to.

Saliva samples:

- In total we will ask your child to provide 5 saliva samples throughout the day; the first sample should be taken from him/her before you set off for your visit to our research centre. The next sample will be collected when you arrive at the centre, and the next two samples will be taken just before and after the MRI scan. These samples will be used to tell us about your child's stress hormone levels.

- A final saliva sample will be collected from you and your child for DNA analysis. These samples will allow us to look at the different types of genes involved in people's reaction to stress. With this information, we will be able to see if the relationship between a pregnant mother's emotions and her child's development differs depending on what 'stress genes' she or her child have.

All the results will be anonymised, so you and your child will not be identified on our computers by name, but by numbers, and all information will be strictly confidential. The DNA samples will not be kept after we analyse them, and, again, data will not be connected to your names.

Brain Scanning

The second part of the day will involve your child having a brain scan using **MRI - Magnetic Resonance Imaging.**

The MRI scanner is a long machine that looks a bit like a tube. Before the scan, your child will be given plenty of time to get used to the way the scanner looks and how it feels to lie down inside it. The tube is open at both ends all the time, and in order to be scanned your child lies on a table that slides into the tube. The MRI machine takes pictures of the brain therefore it is important that your child lies very still so we can get the best pictures possible. Your child will be told that the MRI scanner makes some loud noises during the scanning, and that these are nothing to worry about.

- Once your child is happy to lie comfortably in the scanner, we will give him/her a test to do that is similar to a computer game, but not quite as exciting. Your child will be shown some letters on a screen, and he/she will be asked to press a button when certain letters appear, but not others. This lasts about 10 minutes.

- Finally, your child will be asked to lie back and relax inside the scanner for around 10-15 minutes, while the MRI machine takes some more scans. During the scan your child will be able to take breaks if he/she needs to.

The whole scan will take about 45 minutes depending on whether your child needs any breaks. While your child is lying in the scanner, the person conducting the study will be on the other side of the screen to make sure everything is running smoothly.

Finally, please note that you will be compensated for you and your child's time (£50.00 of high street shopping vouchers), plus reimbursement for your lunch (£6.00). We will also arrange for you paid transport by taxi to and from the test centre.

Safety issues

If your child has any **metal** pieces in his/her body then your child **should not** go into the scanning machine. For example, your child **must not have a scan if** he/she:

- has received **metal injuries to the eye** (caused by metal objects, for example by using a welder)
- has had **metallic objects (including clips)** inserted into their body during an operation
- has a **heart pacemaker**
- has ever received a **shotgun** injury

You will be able to accompany your child into the scanning room to keep him/her company during the scan, but **only if you do not have any metal pieces in your body and have not experienced any of the above**. As the scanner contains a strong magnet, the whole area around the machine needs to be free from metal objects.

The radiographer will go over a list of possible risks with you before you and your child go into the scanning room. MRI scans **do not** involve any radiation. They are **not** the same as x-ray machines.

There is a microphone inside the scanner so that your child can talk to us at any time that they like. If he/she feels slightly fearful at being in an enclosed space we can stop the process of scanning immediately. The other thing he/she might find uncomfortable is the level of machine noise, although most children tend to get used to it after a while. If we are unable to complete the testing on the day, we may offer to complete the remaining tests on a second visit. Of course, it is entirely up to you whether you choose to return, you are under no obligation to do so.

Remember that you and/or your child are free to withdraw from the study at any time without giving a reason. This will not affect your child's current or future medical treatment in any way.

In the unlikely event of us finding any unexpected abnormalities or you suffering from the process (physical or psychological) we will ask your permission to contact yours and/or your child's GP.

What if something goes wrong?

In the unlikely event of your child suffering any adverse effects as a consequence of your participation in this study, you will be compensated through King's College London's 'No-fault Compensation Scheme'.

Thank you very much for reading this information. If you are worried or need further information about any aspect of this study please contact Dr. Michael Craig 0207 848 0364, or write us at the Section of Brain Maturation, PO50, Institute of Psychiatry, De Crespigny Park, London SE5 8AF.

This research is funded by the Baily Thomas Charitable Fund.

The study has received a favourable opinion from the Brent Research Ethics Committee: REC number 09/H0717/39.

Appendix 22: Stressful Life Events Questionnaire

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Appendix 23: Consent form – Study 2

Version 3. 01/08/2010 BRENT NHS REC REC number 09/H0717/39

Institute of Psychiatry
at The Maudsley

Section of Brain Maturation
Division of
Psychological Medicine

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Pregnancy and child development: A brain imaging study

PRINCIPAL INVESTIGATOR: Dr. Michael Craig

Child's Name: ……

The mother should complete the whole of this sheet herself on behalf of her child.

The study has been explained to me by:

Prof/Dr/Mr/Mrs/Ms ……

Please tick each statement as applicable:

I confirm that I have read and understood the information sheet for the above study, and have had the opportunity to ask questions. ☐

I understand what is required from me and my child to participate in this study. ☐

I am willing to fill in questionnaires about how I am feeling and about my child's development. ☐

I am willing to collect saliva samples from myself and my child. I understand that the samples will be used for gene analysis as described in the information sheet given to me. ☐

I agree to my child having an MRI scan. ☐

I understand that our participation is voluntary and that I am free to withdraw at any time, without giving reason, and without my medical care being affected. ☐

I understand that my records and those of my child may be accessed by responsible individuals from Imperial College and Kings College for audit purposes only. ☐

I agree to my child's GP being informed of his/her participation in this study. ☐

Signed:…… Date :……

NAME IN BLOCK CAPITALS: ……

Relationship to child: ……

Investigator's signature:…… Date :……

NAME IN BLOCK CAPITALS: ……

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